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<b>(21) International Application Number:</b> PCT/US97/01785 <b>(22) International Filing Date:</b> 29 January 1997 (29.01.97) <b>(30) Priority Data:</b> 60/010,795      29 January 1996 (29.01.96)      US 08/597,936      7 February 1996 (07.02.96)      US Not furnished      29 January 1997 (29.01.97)      US  <b>(60) Parent Application or Grant</b> (63) Related by Continuation US      Not furnished (CIP) Filed on      Not furnished  <b>(71) Applicants (for all designated States except US):</b> UNIVERSITY OF MARYLAND AT BALTIMORE [US/US]; 520 Lombard Street, Baltimore, MD 21201 (US). USBIOMATERIALS CORPORATION [US/US]; One Progress Boulevard, Box #23, Alachua, FL 32615 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> LITKOWSKI, Leonard, J. [US/US]; 621 Sussex Road, Baltimore, MD 21286 (US). HACK, Gary, D. [US/US]; 8908 Skyrock Court, Columbia,	MD 21046 (US). GREENSPAN, David, C. [US/US]; 3116 N.W. 62nd Terrace, Gainesville, FL 32606 (US).  <b>(74) Agents:</b> GRUDZIECKI, Ronald, L. et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>	
<b>(54) Title:</b> BIOACTIVE GLASS COMPOSITIONS AND METHODS OF TREATMENT USING BIOACTIVE GLASS  <b>(57) Abstract</b> <p>A novel silica-based bioactive glass composition that can be used in conjunction with a delivery agent such as a toothpaste, gel, etc., having a particle size range &lt; 90 <math>\mu\text{m}</math> which will form a rapid and continuous reaction with body fluids due to the immediate and long-term ionic release of Ca and P from the core silica particles, to produce a stable crystalline hydroxy carbonate apatite layer deposited onto and into the dentin tubules for the immediate and long-term reduction of dentin hypersensitivity and tooth surface remineralization.</p>		

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BIOACTIVE GLASS COMPOSITIONS AND  
METHODS OF TREATMENT USING BIOACTIVE GLASS

This application is a continuation-in-part application of copending U.S. Application Serial No. 08/597,936 filed February 7, 1996, the disclosure of which is hereby incorporated by reference. This application is further a continuation-in-part application of copending U.S. Provisional Application Serial No. 60/010,795 filed January 29, 1996, the disclosure of which is hereby incorporated by reference.

### FIELD OF THE INVENTION

The present invention relates to bioactive glass compositions. More particularly, the present invention relates to improved compositions of bioactive glass including particles having combinations of size ranges significantly lower than previous compositions. The present invention also relates to various methods of treatment including the use of such bioactive glass compositions.

### BACKGROUND OF THE INVENTION

Human tooth enamel naturally undergoes a process of demineralization. Exposure of enamel to saliva and food slowly leaches minerals from teeth and eventually leads to increased susceptibility to decay. This process of demineralization results in incipient caries which are typically very small defects in the enamel surface that are thus far usually left untreated. Carious dentin demineralization also may occur in patients that have exposed regions of dentin resulting from decay below the cementum-enamel junction. Accordingly, there has been much work associated with slowing this natural process of demineralization including the application of fluoride and other topical treatments.

For example, U.S. Patent No. 5,427,768 discloses calcium phosphate solutions which are supersaturated with respect to calcium phosphate solids and carbon dioxide. The solutions deposit calcium phosphate compounds with or without fluoride on and in the tooth weaknesses such as dental caries, exposed root, or dentin. U.S. Patent Nos. 5,268,167 and 5,037,639 disclose the use of amorphous calcium compounds such as amorphous calcium phosphate, amorphous calcium phosphate fluoride and amorphous calcium carbonate

phosphate for use in remineralizing teeth. These amorphous compounds, when applied to \_  
dental tissue prevent and/or repair dental weaknesses. The disadvantages of these methods  
include (1) a low pH necessary for the application which can be an irritant, (2) rapid reaction  
results in a very short term effect, (3) since these methods use solutions, the actual reactions  
5 are difficult to control from patient to patient, and (4) since the reactions are rapid and of  
short duration, the procedure must be repeated to maintain the effect. Also, both methods  
require maintaining at least one solution with pressurized CO<sub>2</sub> prior to mixing delivery which  
makes the method difficult to incorporate into an over-the-counter procedure.

Demineralization eventually leads to cavitation of enamel coating such that there is  
10 exposure of the underlying tooth structure. Typically, this type of decay is treated by drilling  
out the decayed region and inserting a semi-permanent filling material. However, there is a  
need for a less invasive means of arresting and reversing decay.

Prophylactic pit and fissure sealants have become widely used in preventing decay in  
areas that are particularly at risk for decay. These sealants have included polymer or other  
15 cements that require a dry application and the use of a fixing agent. These sealants are  
temporary and do not provide for an optimal seal.

Liners and bases are materials that are used to treat newly exposed tooth surfaces such  
as those surfaces exposed by drilling. After a cavity is prepared, it is common practice to  
apply a liner or base before filling the cavity with a filling material. A liner is a thin coating  
20 of material and a base is a thicker coating. Liner and base materials are designed to decrease  
permeability of dentin at the tooth material interface and protect against microleakage around  
and through the fill material and to seal dentin tubules. Earlier liners or "cavity varnishes"  
include materials such as organic "gums" dissolved in organic solvents. Upon evaporation of  
the organic solvent, the gum is left behind. Disadvantages associated with these organic  
25 gums are well documented and include leaky junctions, lack of adherence, acid vulnerability,  
etc. Another method of lining is disclosed in U.S. Patent No. 4,538,990 which describes  
applying a 1 to 30% w/v neutral oxalate salt solution, such as dipotassium oxalate to the  
smear layer and then applying a 0.5 to 3% w/v of an acidic oxalate salt solution such as  
monopotassium monohydrogen oxalate to the layer. Research has shown poor seal occlusion  
30 of the tubules with this method.

U.S. Patent No. 5,296,026 discloses glass phosphate cement compositions and methods for their use as surgical implant materials to fill cavities in bone and canals in teeth. The cement compositions include  $P_2O_5$ , CaO, SrO and  $Na_2O$  in combination with an aqueous liquid with or without therapeutic agents. Mixing the powder and liquid results in a hardening reactions. When the cement is implanted into hard tissue, it serves as a filler/graft material and along with the release of leachable constituents it can assist in the healing and maintenance of healthy bone.

Various bioactive and biocompatible glasses have been developed as bone replacement materials. Studies have shown that these glasses will induce or aid osteogenesis in a physiologic systems. Hench et al, *J. Biomed. Mater. Res.* 5:117-141 (1971). The bond developed between the bone and the glass has been demonstrated to be extremely strong and stable. Piotrowski et al., *J. Biomed. Mater. Res.* 9:47-61 (1975). Toxicology evaluation of the glasses has shown no toxic effects in bone or soft tissue in numerous in vitro and in vivo models. Wilson et al., *J. Biomed. Mater. Res.* 805-817 (1981). The glass has been reported to be bacteriostatic or bacteriocidal most likely related to the change in pH induced by the dissolution of the ions from the surface of the glass and lack of bacterial adherence to the glass surface. Stoor et al, *Bioceramics* Vol. 8 p. 253-258 Wilson et al (1995).

The bonding of the glass to bone begins with the exposure of the glass to aqueous solutions.  $Na^+$  in the glass exchanges with  $H^+$  from the body fluids causing the pH to increase. Ca and P migrate from the glass forming a Ca-P rich surface layer. Underlying this Ca-P rich is a layer which becomes increasingly silica rich due to the loss of Na, Ca and P ions (U.S. Patent No. 4,851,046).

The behavior of the bioactive glass as solid implants in a dental application was reported by Stanley et al., *Journal of Prosthetic Dentistry*, Vol. 58, pp. 607-613 (1987). Replicate tooth forms were fabricated and implanted into extracted incisor sockets of adult baboons. Successful attachment of the implants to surrounding bone was seen after histologic examination at six months. Clinical application of this technique is presently available for human use. Endosseous Ridge Maintenance Implant ERMI®. Particulate bioactive glass has been used for periodontal osseous defect repair (US Patent No. 4,851,046) utilizing a size range of 90-710  $\mu m$  and a compositional range described in the following chart.

## Component Weight Percentage

	SiO <sub>2</sub>	40-55
	CaO	10-30
	Na <sub>2</sub> O	10-35
5	P <sub>2</sub> O <sub>5</sub>	2-8
	CaF <sub>2</sub>	0-25
	B <sub>2</sub> O <sub>3</sub>	0-10

Previously described data has shown that 60% silica is beyond the limit of bioactive melt derived glasses. Okasuki et al. Nippon Seramikkusu Kyokai Gakijutsu Konbuski, Vol. 99, pp. 1-6 (1991).

The 90-710  $\mu\text{m}$  size range was determined to be the most effective for periodontal applications when in direct contact with bone. However, size ranges smaller than 90  $\mu\text{m}$  were ineffective due to their high rate of reactivity and rapid resorption at the bony site. Moreover, size ranges smaller than 90  $\mu\text{m}$  were determined to be ineffective in soft tissue sites also due to the presumption that the smaller particles were removed by macrophages (see U.S. Patent No. 4,851,046). A size range of less than 200  $\mu\text{m}$  was also found to be ineffective in certain bone defects (see U.S. Patent No. 5,204,106) due to the high rate of reactivity.

U.S. Patent No. 4,239,113 ("the '113 patent") also describes the use of a bone cement. The '113 patent only discloses bioactive glass ceramic powder having a particle size of 10-200 microns. Moreover, the '113 patent also requires the use of methylmethacrylate (co)polymers and vitreous mineral fibers.

None of the foregoing methods or compositions provide for the combined advantages of both easy application and adherence to tooth structure including penetration into very small tooth structure defects and the opportunity for continued chemical and physical interaction with tooth structure after application.

Accordingly, it is an object of the present invention to provide a composition capable of chemical and physical interaction with tooth structure that is easily applied and readily adherent to tooth structure.

It is a further object of the invention to provide a method of using such a bioactive glass composition to treat a variety of dental and other conditions.

### SUMMARY OF THE INVENTION

5 The present invention relates to, for example, a bioactive glass composition including particulate bioactive and biocompatible glass including by weight percentage:

	SiO <sub>2</sub>	40-60
	CaO	10-30
	Na <sub>2</sub> O	10-35
	P <sub>2</sub> O <sub>5</sub>	2-8
10	CaF <sub>2</sub>	0-25
	B <sub>2</sub> O <sub>3</sub>	0-10
	K <sub>2</sub> O	0-8
	MgO	0-5,

15 the particulate bioactive and biocompatible glass including particles less than 90 µm and an effective remineralizing amount of particles less than about 10 µm. The present invention also relates to various methods of dental treatment including remineralization, sealing fissures and/or pits, lining tooth structure, treating decay, capping pulp, treating sensitive post surgical tooth structure, sealing dentinal tubules, and surface for tissue regeneration.

### 20 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a dentin control surface that has been treated with 37% phosphoric acid for 30 seconds to remove any smear layer after sectioning and grinding to emulate clinical sensitivity. The surface has not been treated with bioactive glass in accordance with the  
25 present invention (2000X magnification).

Figure 2 is a dentin control surface that has been treated with 37% Phosphoric acid for 30 seconds to remove any smear layer after sectioning and grinding to emulate clinical sensitivity. The surface has not been treated with bioactive glass in accordance with the present invention (3000X magnification).



Figure 3 is a dentin surface that has been treated with an acid etch and treated with a bioactive glass composition in accordance with the present invention in water and glycerin for 2 minutes (Particle size range submicron to 90  $\mu\text{m}$ , 1000X magnification).

5 Figure 4 is a dentin surface that has been acid etched and subsequently treated with a bioactive glass composition in accordance with the present invention in water and glycerin for 2 minutes. The surfaces were subsequently agitated and water rinsed for 2 minutes (Particle size range submicron to 20  $\mu\text{m}$ , 2000X magnification).

10 Figure 5 is a dentin surface that has been acid etched and subsequently treated with a bioactive glass composition in accordance with the present invention and placed in water for 3 days. There was no subsequent agitation, but the surface was water rinsed for 2 minutes (Particle size range submicron to 90  $\mu\text{m}$ , 2000X magnification).

15 Figure 6 is a dentin surface that has been acid etched and subsequently treated with a bioactive glass composition in accordance with the present invention in water and toothpaste for 2 minutes with agitation and a subsequent 2 minute water rinse (Particle size range submicron to 3  $\mu\text{m}$ , 3000X magnification).

20 Figure 7 is a dentin surface that has been acid etched and treated with a bioactive glass composition in accordance with the present invention in water and toothpaste for 2 minutes with agitation and water rinse for 2 minutes (Particle size range submicron to 3  $\mu\text{m}$ , 3500X magnification).

25 Figures 8 and 9 each include a dentin surface which has been acid etched with phosphoric acid, treated with a bioactive glass in accordance with the present invention for 2 minutes and immersed in a phosphate buffered saline for 5 days (Particle size range submicron).

Figure 10 depicts a dentin surface that has been acid etched and subsequently treated with a single application of a bioactive glass composition in accordance with the present invention.

Figure 11 depicts a dentin surface that has been acid etched and treated with three separate applications of a bioactive glass composition in accordance with the present invention.

30 Figure 12 is a Fourier Transform Spectroscopy (FTIR) performed on samples treated with optimal sizes and shaped particulate bioactive glass.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a bioactive glass composition which is useful in, for example, enamel remineralization, incipient caries remineralization, carious dentin  
5 remineralization, caries prevention, arresting decay, reversing decay, anti-caries, pit and fissure sealants, prophylactic pastes, fluoride treatments, dentinal sealants, etc. It can also be included in toothpastes, liners, bases, gels, and restorative material e.g. packing, indirect pulp capping agent, etc. Compositions in accordance with the present invention are also useful in the treatment of surfaces after periodontal surgery to decrease dentinal sensitivity and  
10 enhance tissue attachment. The compositions are active in treating various defects associated with a variety of dental and other conditions and actually chemically and physically bond to the tooth thereby remineralizing tooth structure.

As referred to herein, remineralization is the formation of hydroxyapatite. The formation of hydroxyapatite begins with exposure of a bioactive glass composition to  
15 aqueous solutions. It is believed that the sodium ions ( $\text{Na}^+$ ) in the bioactive glass exchanges with  $\text{H}^+$  ions in body fluids causing pH to increase. Calcium and phosphorus then migrate from the bioactive glass forming a calcium-phosphorous rich surface layer. An underlying silica rich zone slowly increases as the sodium ion in the bioactive glass continues to exchange with the hydrogen ion of the solution. After time, the calcium-phosphorous rich  
20 layer crystallizes into a hydroxyapatite material. Collagen can become structurally integrated with the apatite agglomerates. As hereinafter referred to, an effective remineralizing amount is any amount capable of forming hydroxyapatite.

As the term "a tooth structure" is used herein, it is intended to refer to any feature or features of a tooth including but not limited to enamel, dentin, pulp, tooth root structure,  
25 cementum, root dentin, coronal dentin, any dental manufacture, etc.

A bioactive glass in accordance with the present invention is a glass composition that will form a layer of hydroxycarbonate apatite *in vitro* when placed in a simulated body fluid. For example, the following composition by weight will provide a bioactive glass:

	SiO <sub>2</sub>	40-60
	CaO	10-30
	Na <sub>2</sub> O	10-35
	P <sub>2</sub> O <sub>5</sub>	2-8
5	CaF <sub>2</sub>	0-25
	B <sub>2</sub> O <sub>3</sub>	0-10
	K <sub>2</sub> O	0-8
	MgO	0-5

Bioactive glasses with these properties provide a more efficacious material for interaction with the tooth structure. A biocompatible glass in accordance with the present invention is one that does not trigger an overwhelmingly adverse immune response.

In accordance with the present invention, it has been found that bioactive glasses of specified particle sizes are particularly useful in treating the above-mentioned conditions. Specifically, surprising results are obtained by the present invention where small and very small particles are combined. For example, when compositions including small particles that are capable of bonding with tooth structure (e.g. less than about 90 microns) as well smaller particles (e.g. less than about 10) are used in combination, the larger of these particles adhere to tooth structure and act as ionic reservoirs while the smaller are capable of entering and lodging inside of various tooth structure surface irregularities. The larger of these particles provide a reservoir of additional calcium and phosphorous so that the mineralization, or depositing of the calcium phosphate layer begun by the small particles can continue. Additional calcium and phosphorous can be leached to all tooth structure as well as to particles which have become attached to the inside or at the openings of surface irregularities of tooth structure such as dentinal tubules. This in turn provides for continuation of the entire reaction and continued growth of the smaller of these particles which have lodged inside or over the openings of such surface irregularities and can result in effectively coating or filling the surface irregularity. This excess concentration of ions of calcium and phosphorous is necessary for continued reaction of the smaller of these particles to take place because the smaller particles quickly exhaust their ions as a result of their relatively high surface area. The larger of these particles will react and release their ions more slowly as a longer term effect. Furthermore, the larger of these particles will mechanically abrade the tooth surface

opening various surface irregularities allowing small particles to enter and react with the surface irregularity.

5 This effect is very beneficial in a variety of applications. For example, in preventing caries or decay, the composition of the present invention is capable of penetrating into the depths of the smallest of surface irregularities and receiving a continued supply of ions from larger nearby particles so that it is able to grow after exhausting its stored ion supply. This is also very useful in sealing pits and fissures and a much more effective and long lasting seal is obtained.

10 In some embodiments of the present invention, extremely small particles are used. For example, particles that are in the range of 2  $\mu\text{m}$  to submicron fit inside dentin tubules that are approximately 1-2  $\mu\text{m}$  in diameter. The occlusion of these tubules leads to a significant reduction in the amount of sensitivity after, for example, periodontal surgery. Preferably, a mixture of particles less than two microns and larger than 45 microns in diameter are used. It has been found that this combination yields a particularly effective composition.

15 Compositions in accordance with the present invention generally do not require time to set. Previous compositions were easily washed away by mechanical abrasion caused by brushing, exposure to mild acids in food, salivary flow or other liquids which normally come in contact with the teeth. However, some compositions in accordance with the present invention have been able to generally withstand significant agitation, rinsing with water and long term soaking in simulated saliva for five days. Moreover, many of the small particles of the present invention do not require a set time because they begin to chemically react and adhere to tooth structure as soon as they come into contact with these surfaces and fluids naturally present in the mouth. Although compositions in accordance with the present invention are effective with a single application, it is likely that multiple applications will be more efficacious.

25 Surprisingly, the relatively small bioactive particulate glass of the present invention does not generate a significant immune response. Moreover, it is generally not engulfed by macrophages and rendered inactive in this application.

30 The composition of the present invention is capable of providing a bioactive layer that will form a new structural layer which is a lasting remineralization of tooth structure. This has been verified by the reformation of a hydroxycarbonate apatite layer on dentin surfaces

after treatment with compositions in accordance with the present invention with Fourier Transform Infrared spectroscopy (FTIR).

In one embodiment in accordance with the present invention, the particles have a particle size of about 20 microns with about 30 percent of the particles less than 10 microns.

5 In another embodiment in accordance with the present invention the particles have an average particle size of 10 microns with at least 25% smaller than 2 microns.

The compositions of the present invention may be formulated into toothpaste. In fact, the particles may replace the silica currently used in toothpastes. The addition of fluoride in the glass composition will enhance and strengthen the tooth structure. In addition to direct  
10 application of the bioactive glass to the teeth, the bioactive glass composition of the present invention can also be applied in a saline or distilled water based medium.

The compositions of the present invention may also be formulated into mouthwash, gel or they may be applied by a dentist as a paste.

### 15 Examples

The following working examples are non-limiting:

*In vitro* experiments were performed using a standardized slab of human tooth dentin from extracted teeth. These discs were cut from the extracted teeth using an Isomet diamond saw (Buchler Ltd.). The discs were 1.0 mm thick and the size of the tooth. The occlusal  
20 surfaces were ground on a series of wet silicon-carbide papers ranging from 320 to 600 grit. This was done to standardize the test surfaces. The surfaces were treated with 37% phosphoric acid for 60 seconds to remove the smear layer created during the grinding process and open and enlarge all the dentin tubules (See Figures 1 and 2). The surface was rinsed with distilled water for 20 seconds and dried with a stream of oil free air. Each slab was split  
25 in half and the experimental material placed on one-half of the specimen as described in the examples. An untreated slab with open and enlarged tubules is shown in Figures 1 and 2.

Scanning electron microscopy was performed on the slab surface in each group. The slabs were mounted on scanning electron microscope stubs using silver paste. All specimens were vacuum dried, sputter coated and examined in a JEOL-T200 scanning electron  
30 microscope.

EXAMPLE 1

The starting product was a mixture containing (% by weight)

	SiO <sub>2</sub>	45
5	CaO	24.5
	Na <sub>2</sub> O	24.5
	P <sub>2</sub> O <sub>5</sub>	6

The mixture was melted in a covered platinum crucible at 1350° C for 2 hours to achieve homogenization. The mixture was later quenched in deionized water at 0°C. Fritted glass was placed in an appropriate milling apparatus including ball mill, impact mill. The glass is milled for 2 hours and separated into appropriate size ranges.

The particle size range less than 90 µm was obtained using this process and confirmed by scanning electron microscopy and laser light scattering technique (Coulter LS 100). These mixtures were placed on the dentin slabs previously described.

The exposure times to the dentin varied between two minutes with scrubbing to 3 days with no agitation. The occlusion of the tubules is depicted in Figures 3-7. Visible in Figures 3-7 are total and partial occlusion of the dentin tubules with multiple size of small (1-5 µm) particles present. In addition, larger particles that are visible that will act as reservoirs for the chemical composition. Early formation of hydroxyapatite crystals is beginning on the dentin surface confirmed by FTIR.

EXAMPLE 2

Figures 8 and 9 indicate the results obtainable by using submicron particles made in accordance with Example 1. The samples of figures 8 and 9 are dentin surfaces which have been acid etched with phosphoric acid, treated with a bioactive glass for 2 minutes and immersed in a phosphate buffered saline for 5 days. With the lack of large particles for reservoir activity, there was less complete regeneration as confirmed by FTIR.

### EXAMPLE 3

Example 3 was conducted to illustrate the benefits associated with multiple applications of compositions in accordance with the present invention. First, an acid etched dentin surface was treated with a single treatment of bioactive particulate glass for two minutes and is depicted in Figure 10. A dentin surface which has been acid etched and treated three times for two minutes is depicted in Figure 11.

Figure 10 shows significant penetration and occlusion of the tubules with a bonding over the surface of the dentin. There are not many large particles visible in Figure 10. In Figure 11, there is even more significant penetration and occlusion of the tubules and a greater number of particles present. This demonstrates the benefits associated with multiple application including the tubules as well as increased presence of larger reservoirs of Ca and P ions. This also demonstrates interparticle welding of the larger particles to the smaller particles already bound to the surface.

### EXAMPLE 4

Example 4 further illustrates the benefits associated with the use of particles less than 2 microns in combination particles greater than 45 microns in size. FTIR spectra for the following samples are included in figure 12 to illustrate remineralization:

Sample No. 1	Control (untreated dentin surface)
Sample No. 2	Acid etched dentin surface
Sample No. 3	Treated with particles of bioactive glass less than 2 microns in particle size for two minutes
Sample No. 4	Treated with particles of bioactive glass wherein 40% were less than 2 microns, 15% were in the range of 8 to 2 microns, 15% were in the range of 8 to 20 microns, 15% were in the range of 20 to 38 microns and 15% were in the range of 38-90 microns.

As illustrated in Figure 12, the control sample provides a representative view of the spectrum of hydroxycarbonate apatite (HCA). The shape of the peaks between wave number

1150 to 500 are very characteristic of HCA. In sample 2, the peaks are disrupted after treatment with the acid etchant, especially in the 1150 to 900 range. This indicates a loss of the mineral components of the tooth structure, Calcium and Phosphorous. Sample 3 shows a partial remineralization of the Ca and P on the tooth structure. Sample 4 was treated with the optimal size and shape mixture of bioactive glass and shows an almost complete remineralization. A photomicrograph of Sample 4 is included as Figure 11.

### EXAMPLE 5

Comparative Example 5 shows the benefits associated with the use of particles less than 10 microns in combination with particles greater than 45 microns in size over the use of just particles less than 2 microns or 53-90 $\mu$ . A control sample of untreated dentin surface was used in addition to treated surfaces as described below:

Number of Applications	Sample Composition	Score	Observations
Single	53-90 $\mu$	2	About 50% occluded tubules with large particles present
	Control	0	No particles present
Single	<2 $\mu$	2	Above 50% closure, no large particles seen
	Control	0	Open tubules
Single	50% 53-90 $\mu$ 50% <2 $\mu$	+3	75%+ tubules occluded
	Control	0	Open tubules
Multiple	53-90 $\mu$	2	Partial closure of tubules with large particles present
	Control	0	Minimal occlusion seen



Multiple	$<2\mu$	2	Partial closure of tubules with small particles present
	Control	0	Minimal occlusion seen
Multiple	50% 53-90 $\mu$ 50% $<2\mu$	+3	Best results-- tubules closed; difficult to find open tubules
	Control	0	Minimal occlusion seen

5 All samples in the above Table were subjected to a moist environment for 24 hours and then dried for 48 hours.

As seen above, the combination of particles less than 2 microns and 53-90 $\mu$  provided the best results. It is believed that the presence of both size ranges permits the smaller particles which have lodged in the tubules to continue growth after they have exhausted their own Ca and P ions and are able to make use of such ions from other nearby larger particles acting as reservoirs of Ca and P ions.

#### OTHER EXAMPLES

15 The composition of the starting product for the following examples was the same as Example 1 except the level of SiO<sub>2</sub> was 45%, 55%, and 60%. Also, the method of preparation was different. The mixture was melted in a covered platinum crucible at 1350°C for 2 hours to achieve homogenization. The mixture was poured into a slab, allowed to cool to room temperature and crushed with a hammer. Crushed glass fractions were then separated by sieving through a standard screen. Fractions were then separated and retained.

The particle size range less than 90  $\mu$ m was obtained using this process and confirmed by scanning electron microscopy and laser light scattering technique (Coulter LS 100). These mixtures were placed on the dentin slabs previously described.

25 Samples containing 45%, 55%, and 60% SiO<sub>2</sub> were utilized in the preparations with the same results seen in Example 1. Again, the key to this data was the presence of the size range of particles. Present in these examples are ranges up to 60% silica with a size range in

particles from submicron to 90 micron showing like reactions to Example 1 on the dentin surfaces. —

Although the present invention has been described in one or more embodiments, this description is not intended to in any way limit the scope of the claims. .

We claim:

1. A bioactive glass composition comprising particulate bioactive and biocompatible glass including by weight percentage:

	SiO <sub>2</sub>	40-60
5	CaO	10-30
	Na <sub>2</sub> O	10-35
	P <sub>2</sub> O <sub>5</sub>	2-8
	CaF <sub>2</sub>	0-25
	B <sub>2</sub> O <sub>3</sub>	0-10
10	K <sub>2</sub> O	0-8
	MgO	0-5,

the particulate bioactive and biocompatible glass including particles less than 90 µm and an effective remineralizing amount of particles less than about 10 µm.

2. A method for preventing tooth decay comprising contacting a tooth structure with the composition of claim 1.

3. A method for treating tooth decay comprising contacting a tooth structure with the composition of claim 1.

4. A method for preventing incipient caries comprising contacting a tooth structure with the composition of claim 1.

5. A method for remineralizing enamel comprising contacting a tooth structure with the composition of claim 1.

6. A method for incipient caries remineralization comprising contacting a tooth structure with the composition of claim 1.

7. A method for sealing fissures in tooth structure comprising contacting a tooth structure with the composition of claim 1.

8. A method for sealing pits in tooth structure comprising contacting a tooth structure with the composition of claim 1.

9. A method for lining tooth structure comprising contacting a tooth structure with the composition of claim 1.

10. A method for capping pulp comprising contacting a tooth structure with the composition of claim 1.

11. A method for treating tooth hypersensitivity comprising contacting a tooth structure with the composition of claim 1.

12. A method for treating tooth structure after periodontal surgery comprising contacting a tooth structure with the composition of claim 1.

13. A composition for treating teeth comprising the composition of claim 1 and a toothpaste, liner, base, gel, restorative material, glycerin gel, mouthwash, prophylactic paste, or indirect pulp capping agent, or mixture thereof.

14. A bioactive glass composition comprising particulate bioactive and biocompatible glass including by weight percentage:

SiO <sub>2</sub>	40-60
CaO	10-30
Na <sub>2</sub> O	10-35
P <sub>2</sub> O <sub>5</sub>	2-8
CaF <sub>2</sub>	0-25
B <sub>2</sub> O <sub>3</sub>	0-10
K <sub>2</sub> O	0-8
MgO	0-5,

the particulate bioactive and biocompatible glass including particles between 45 $\mu$  and 90  $\mu$ m and an effective remineralizing amount of particles less than about 10  $\mu$ m.

15. A method for preventing tooth decay comprising contacting a tooth structure with the composition of claim 14.

16. A method for treating tooth decay comprising contacting a tooth structure with the composition of claim 14.

17. A method for preventing incipient caries comprising contacting a tooth structure with the composition of claim 14.

18. A method for remineralizing enamel comprising contacting a tooth structure with the composition of claim 14.

19. A method for incipient caries remineralization comprising contacting a tooth structure with the composition of claim 14.

20. A method for sealing fissures in tooth structure comprising contacting a tooth structure with the composition of claim 14.

21. A method for sealing pits in tooth structure comprising contacting a tooth structure with the composition of claim 14.

22. A method for lining tooth structure comprising contacting a tooth structure with the composition of claim 14.

23. A method for capping pulp comprising contacting a tooth structure with the composition of claim 14.

24. A method for treating tooth hypersensitivity comprising contacting a tooth structure with the composition of claim 14.

5 25. A method for treating tooth structure after periodontal surgery comprising contacting a tooth structure with the composition of claim 14.

26. A composition for treating teeth comprising the composition of claim 14 and a toothpaste, liner, base, gel, restorative material, glycerin gel, mouthwash, prophylactic paste, or indirect pulp capping agent, or mixture thereof.

10 27. A bioactive glass composition comprising particulate bioactive and biocompatible glass including particles less than 90  $\mu\text{m}$  and an effective remineralizing amount of particles less than about 10  $\mu\text{m}$ .

28. A bioactive glass composition comprising particulate bioactive and biocompatible glass including particles less than 90  $\mu\text{m}$  and an effective remineralizing amount of particles less than about 5  $\mu\text{m}$ .

15 29. A bioactive glass composition comprising particulate bioactive and biocompatible glass including particles less than 90  $\mu\text{m}$  and an effective remineralizing amount of particles less than about 2  $\mu\text{m}$ .

30. A method for preventing tooth decay comprising contacting a tooth structure with the composition of claim 27.

20 31. A method for treating tooth decay comprising contacting a tooth structure with the composition of claim 27.

32. A method for preventing incipient carries comprising contacting a tooth structure with the composition of claim 27.

25 33. A method for remineralizing enamel comprising contacting a tooth structure with the composition of claim 27.

34. A method for incipient caries remineralization comprising contacting a tooth structure with the composition of claim 27.

30 35. A method for sealing fissures in tooth structure comprising contacting a tooth structure with the composition of claim 27.

36. A method for sealing pits in tooth structure comprising contacting a tooth structure with the composition of claim 27.

37. A method for lining tooth structure comprising contacting a tooth structure with the composition of claim 27.

5 38. A method for capping pulp comprising contacting a tooth structure with the composition of claim 27.

39. A method for treating tooth hypersensitivity comprising contacting a tooth structure with the composition of claim 27.

10 40. A method for treating tooth structure after periodontal surgery comprising contacting a tooth structure with the composition of claim 27.

41. A composition for treating teeth comprising the composition of claim 27 and a toothpaste, liner, base, gel, restorative material, glycerin gel, mouthwash, prophylactic paste, or indirect pulp capping agent, or mixture thereof.

15 42. A bioactive glass composition comprising particulate bioactive and biocompatible glass including particles less than 90  $\mu\text{m}$  and particles less than about 2  $\mu\text{m}$ .

43. A method for remineralizing tooth structure comprising contacting a tooth structure in need of remineralization with a bioactive glass composition including an effective remineralizing amount of particles less than 90  $\mu\text{m}$ .

20 44. A bioactive glass composition comprising particulate bioactive and biocompatible glass including by weight percentage:

$\text{SiO}_2$	40-60
$\text{CaO}$	10-30
$\text{Na}_2\text{O}$	10-35
$\text{P}_2\text{O}_5$	2-8
$\text{CaF}_2$	0-25
$\text{B}_2\text{O}_3$	0-10
$\text{K}_2\text{O}$	0-8
$\text{MgO}$	0-5,

25 the particulate bioactive and biocompatible glass including particles between 53 $\mu$  and 90  $\mu\text{m}$   
 30 and an effective remineralizing amount of particles less than about 2  $\mu\text{m}$ .

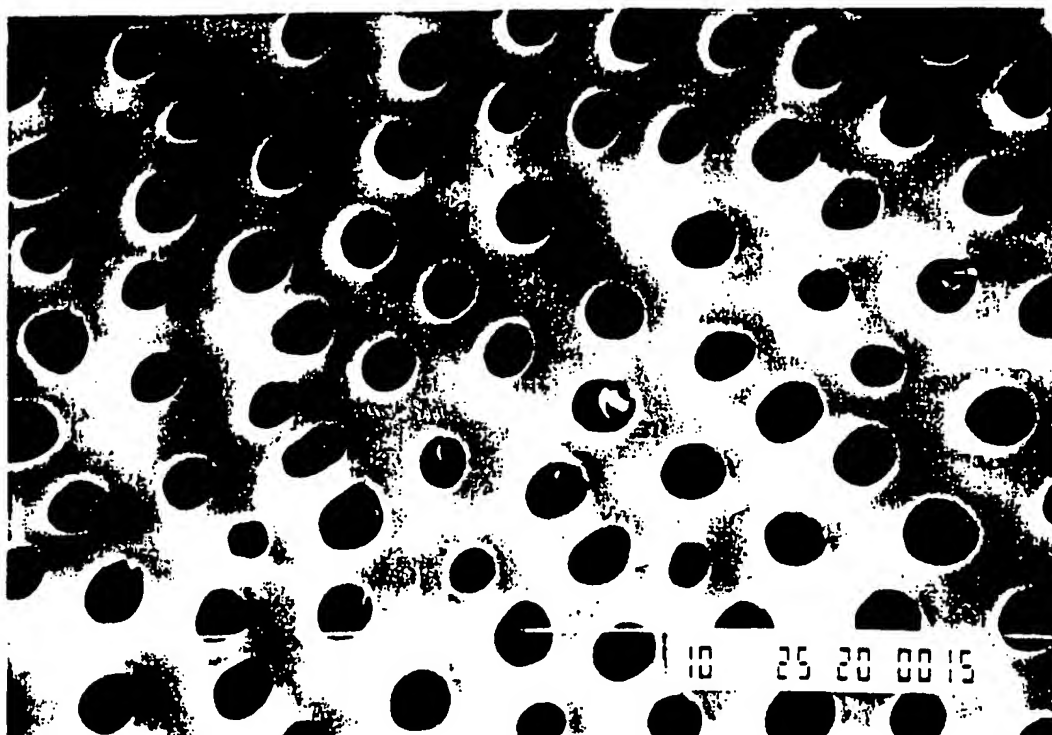


FIG. 1

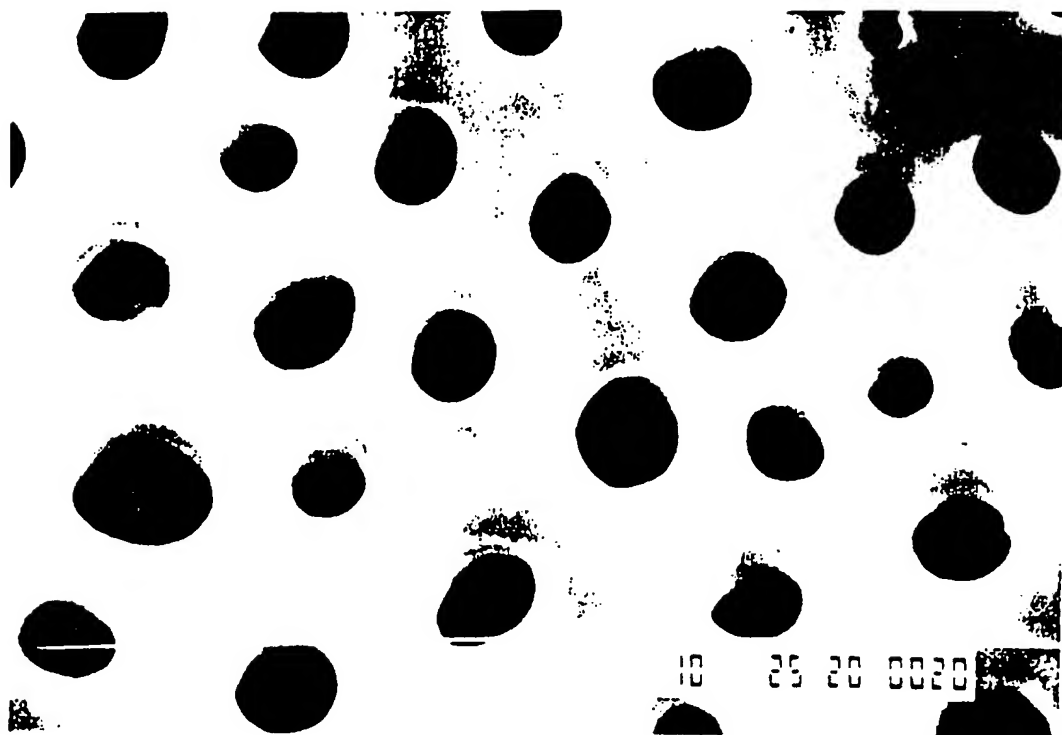


FIG. 2





FIG. 3

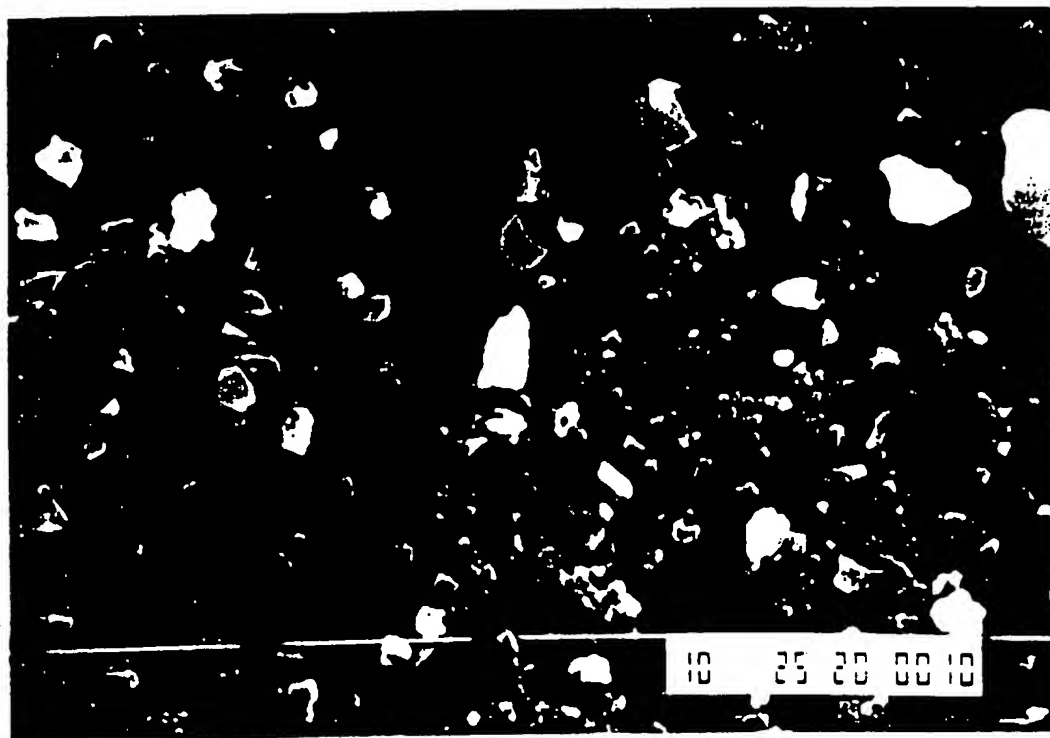


FIG. 4

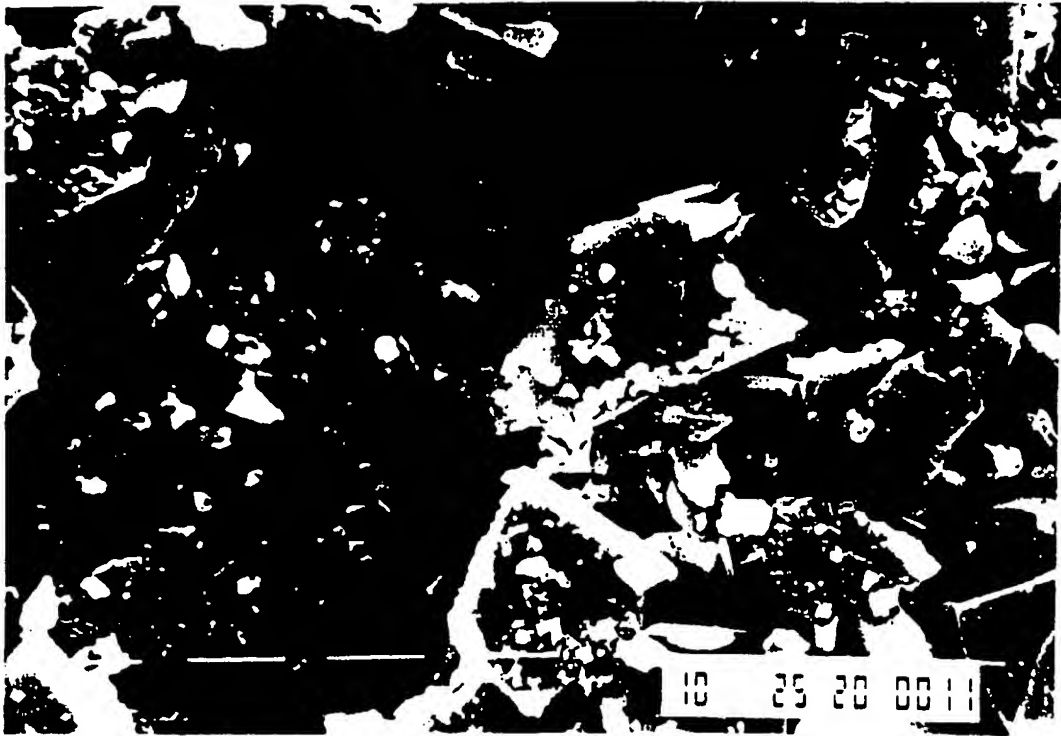


FIG. 5

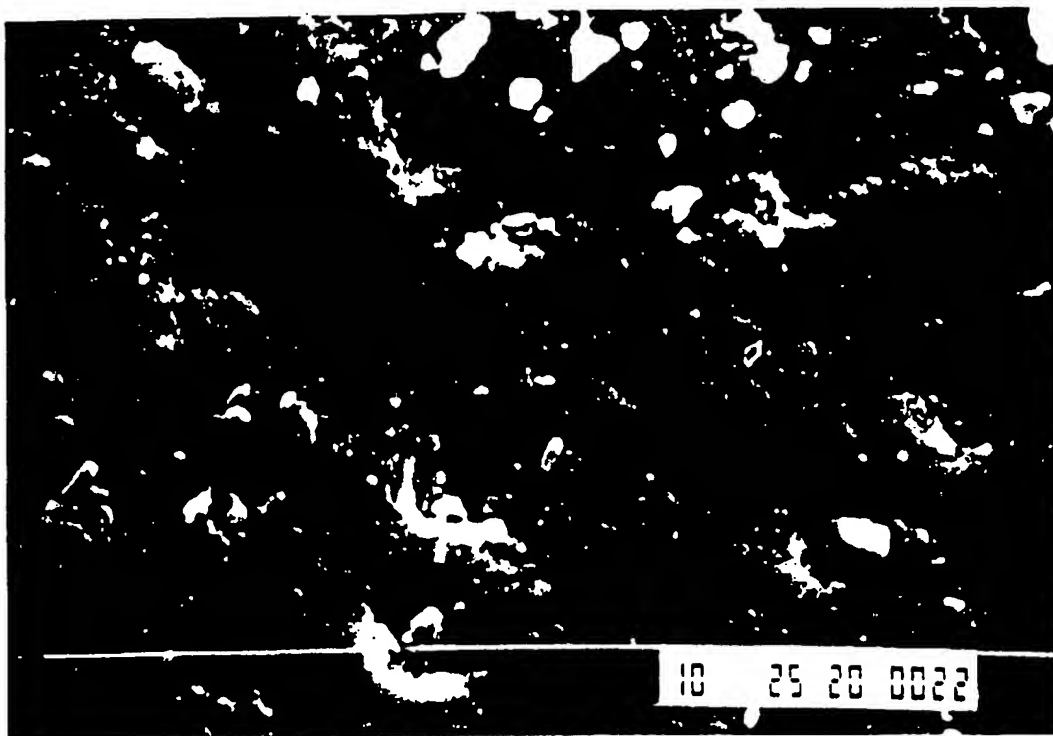


FIG. 6

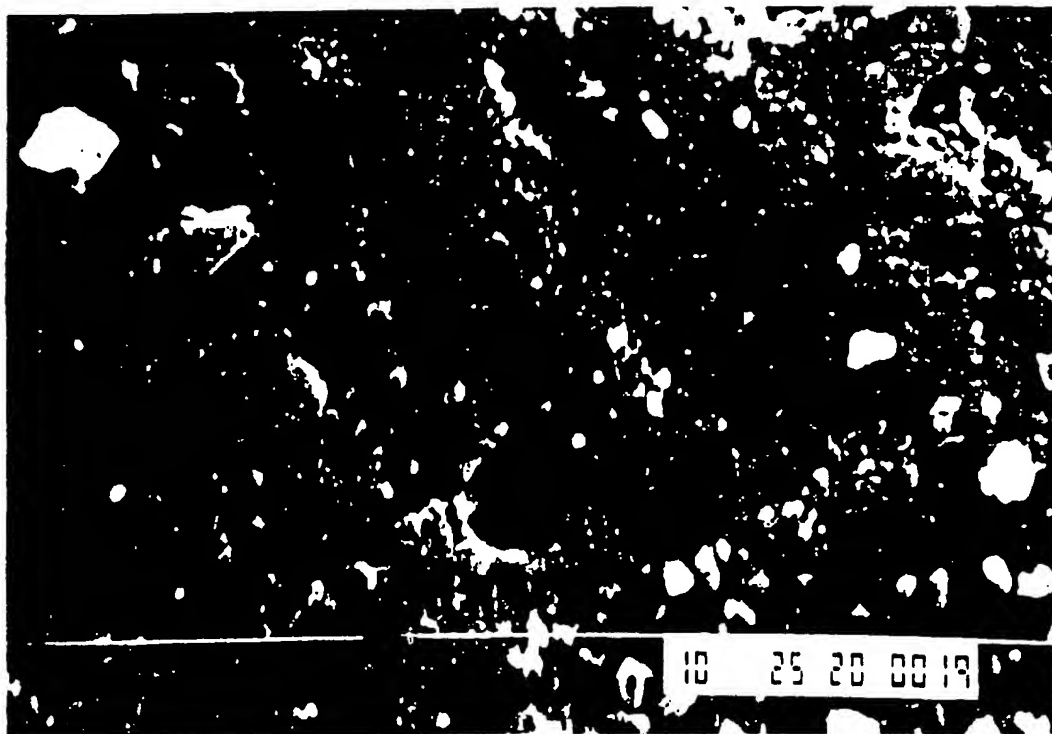


FIG. 7

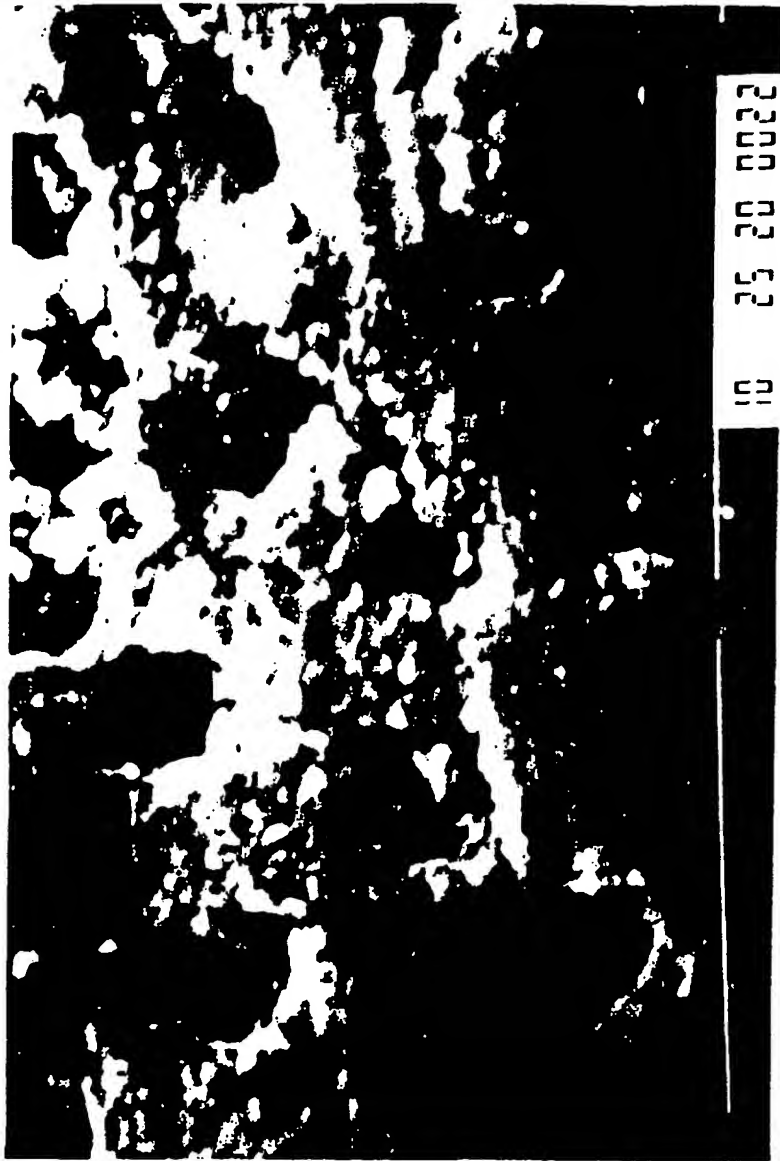


FIG. 8



FIG. 9

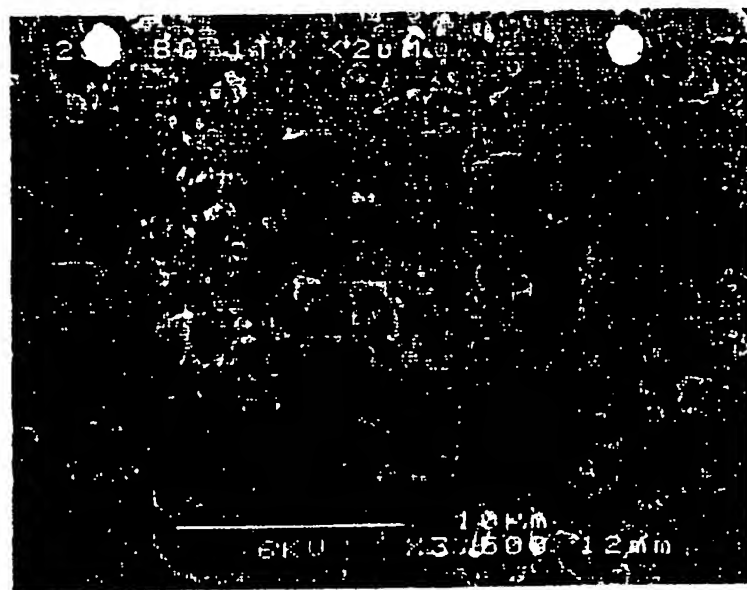


Figure 10

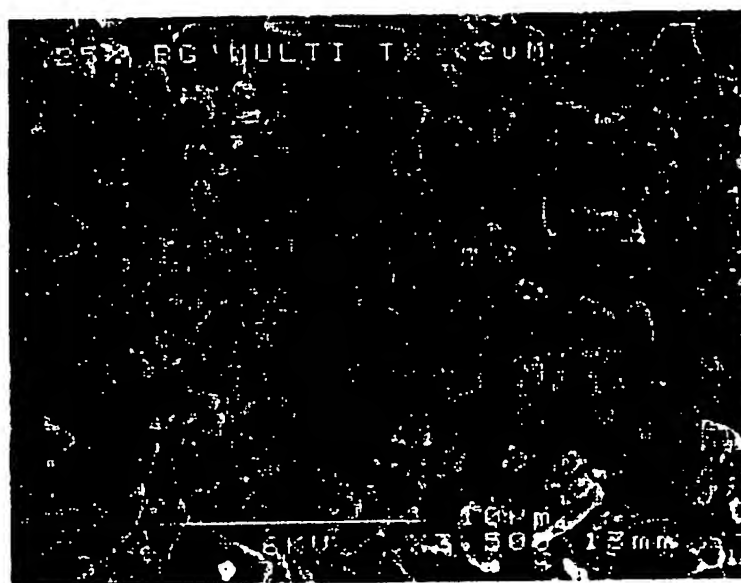


Figure 11



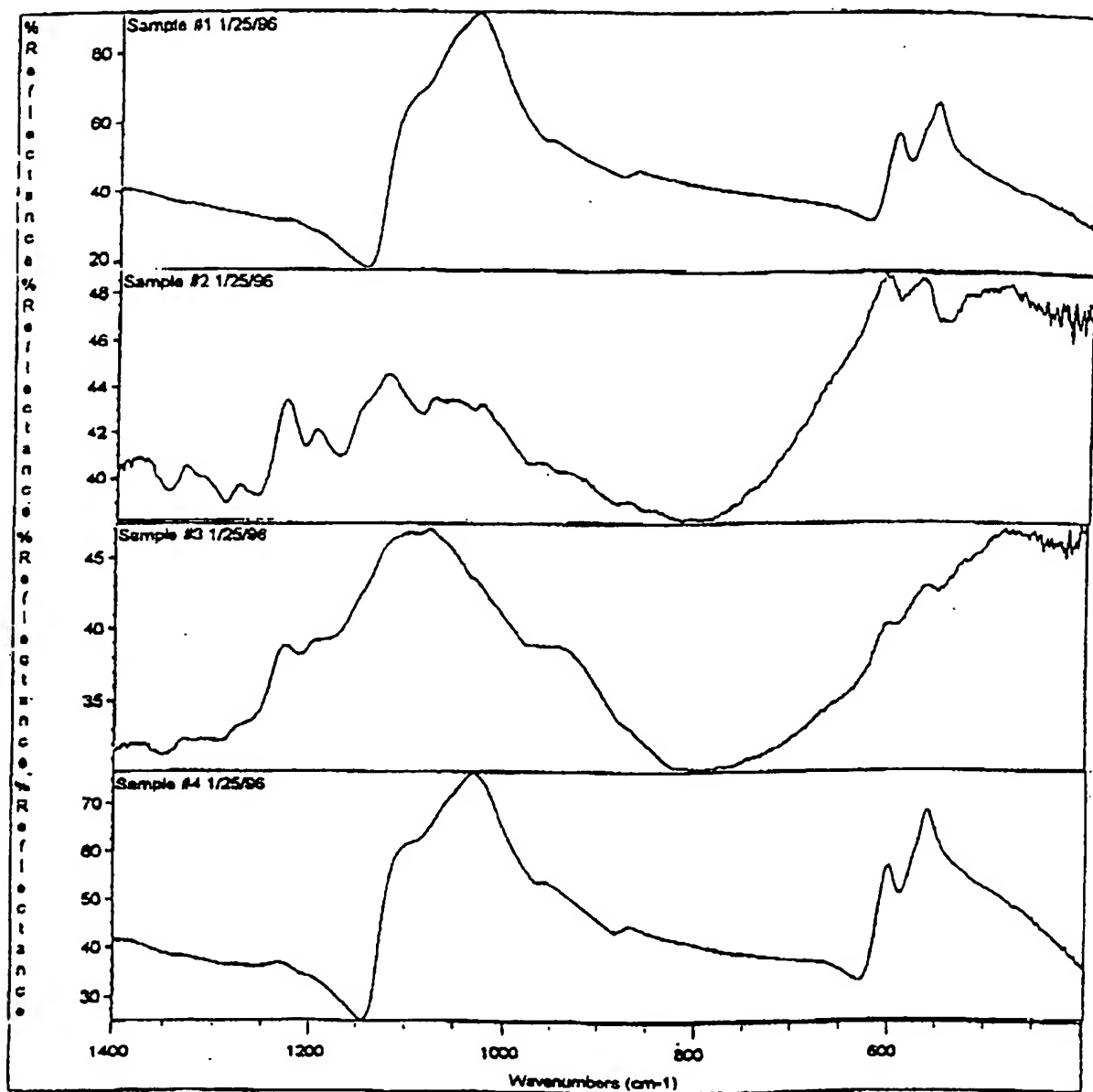


Figure 12

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/01785

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
IPC(6) : CO3C 3/078; A61K 6/027 US CL : 106/35; 501/24, 25, 57, 58, 63, 72; 424/49, 52, 57; 433/226 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) U.S. : 106/35; 501/24, 25, 57, 58, 63, 72; 424/49, 52, 57; 433/226		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,605,415 A (RICHEZ) 12 August 1986, col. 4, lines 47-60.	27-43
X	US 4,783,429 A (SHIBUYA ET AL) 08 November 1988, col. 4, lines 38-39.	27-43
X	US 4,775,592 A (AKAHANE ET AL) 04 October 1988, col. 3, lines 29-36.	27-43
A	US 5,204,106 A (SCHEPERS ET AL) 20 April 1993, col. 3, lines 25-38.	1-44
A	US 4,851,046 A (LOW ET AL) 25 JULY 1989, col. 6, lines 35-43.	1-43
A	US 4,057,621 A (PASHLEY ET AL) 08 November 1977, entire document.	1-43
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Facsimile No. (703) 305-3230	Telephone No. (703) 308-0661	

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/01785

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,239,113 A (GROSS ET AL) 16 December 1980, entire document.	1-43
A	US 5,120,340 A (DUCHEYNE ET AL) 09 June 1992, col. 3, lines 24-62.	1-43
A	US 5,429,996 A (KANEKO) 4 July 1995, entire document.	1-43

**CORRECTED  
VERSION\***

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US97/01785 <b>(22) International Filing Date:</b> 29 January 1997 (29.01.97)  <b>(30) Priority Data:</b> 60/010,795 29 January 1996 (29.01.96) US 08/597,936 7 February 1996 (07.02.96) US Not furnished 29 January 1997 (29.01.97) US  <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US Not furnished (CIP) Filed on Not furnished  <b>(71) Applicants (for all designated States except US):</b> UNIVERSITY OF MARYLAND AT BALTIMORE [US/US]; 520 Lombard Street, Baltimore, MD 21201 (US). USBIOMATERIALS CORPORATION [US/US]; One Progress Boulevard, Box #23, Alachua, FL 32615 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> LITKOWSKI, Leonard, J. [US/US]; 621 Sussex Road, Baltimore, MD 21286 (US). HACK, Gary, D. [US/US]; 8908 Skyrock Court, Columbia,	<b>(74) Agents:</b> GRUDZIECKI, Ronald, L. et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>	
<b>(54) Title:</b> BIOACTIVE GLASS COMPOSITIONS AND METHODS OF TREATMENT USING BIOACTIVE GLASS  <b>(57) Abstract</b>  A novel silica-based bioactive glass composition that can be used in conjunction with a delivery agent such as a toothpaste, gel, etc., having a particle size range < 90 µm which will form a rapid and continuous reaction with body fluids due to the immediate and long-term ionic release of Ca and P from the core silica particles, to produce a stable crystalline hydroxy carbonate apatite layer deposited onto and into the dentin tubules for the immediate and long-term reduction of dentin hypersensitivity and tooth surface remineralization.		

\* (Referred to in PCT Gazette No.44/1997, Section II)

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BIOACTIVE GLASS COMPOSITIONS AND  
METHODS OF TREATMENT USING BIOACTIVE GLASS

This application is a continuation-in-part application of copending U.S. Application Serial No. 08/597,936 filed February 7, 1996, the disclosure of which is hereby incorporated by reference. This application is further a continuation-in-part application of copending U.S.  
5 Provisional Application Serial No. 60/010,795 filed January 29, 1996, the disclosure of which is hereby incorporated by reference.

### FIELD OF THE INVENTION

10 The present invention relates to bioactive glass compositions. More particularly, the present invention relates to improved compositions of bioactive glass including particles having combinations of size ranges significantly lower than previous compositions. The present invention also relates to various methods of treatment including the use of such bioactive glass compositions.

15

### BACKGROUND OF THE INVENTION

Human tooth enamel naturally undergoes a process of demineralization. Exposure of enamel to saliva and food slowly leaches minerals from teeth and eventually leads to  
20 increased susceptibility to decay. This process of demineralization results in incipient caries which are typically very small defects in the enamel surface that are thus far usually left untreated. Carious dentin demineralization also may occur in patients that have exposed regions of dentin resulting from decay below the cementum-enamel junction. Accordingly, there has been much work associated with slowing this natural process of demineralization  
25 including the application of fluoride and other topical treatments.

For example, U.S. Patent No. 5,427,768 discloses calcium phosphate solutions which are supersaturated with respect to calcium phosphate solids and carbon dioxide. The solutions deposit calcium phosphate compounds with or without fluoride on and in the tooth weaknesses such as dental caries, exposed root, or dentin. U.S. Patent Nos. 5,268,167 and  
30 5,037,639 disclose the use of amorphous calcium compounds such as amorphous calcium phosphate, amorphous calcium phosphate fluoride and amorphous calcium carbonate

phosphate for use in remineralizing teeth. These amorphous compounds, when applied to dental tissue prevent and/or repair dental weaknesses. The disadvantages of these methods include (1) a low pH necessary for the application which can be an irritant, (2) rapid reaction results in a very short term effect, (3) since these methods use solutions, the actual reactions are difficult to control from patient to patient, and (4) since the reactions are rapid and of short duration, the procedure must be repeated to maintain the effect. Also, both methods require maintaining at least one solution with pressurized CO<sub>2</sub> prior to mixing delivery which makes the method difficult to incorporate into an over-the-counter procedure.

Demineralization eventually leads to cavitation of enamel coating such that there is exposure of the underlying tooth structure. Typically, this type of decay is treated by drilling out the decayed region and inserting a semi-permanent filling material. However, there is a need for a less invasive means of arresting and reversing decay.

Prophylactic pit and fissure sealants have become widely used in preventing decay in areas that are particularly at risk for decay. These sealants have included polymer or other cements that require a dry application and the use of a fixing agent. These sealants are temporary and do not provide for an optimal seal.

Liners and bases are materials that are used to treat newly exposed tooth surfaces such as those surfaces exposed by drilling. After a cavity is prepared, it is common practice to apply a liner or base before filling the cavity with a filling material. A liner is a thin coating of material and a base is a thicker coating. Liner and base materials are designed to decrease permeability of dentin at the tooth material interface and protect against microleakage around and through the fill material and to seal dentin tubules. Earlier liners or "cavity varnishes" include materials such as organic "gums" dissolved in organic solvents. Upon evaporation of the organic solvent, the gum is left behind. Disadvantages associated with these organic gums are well documented and include leaky junctions, lack of adherence, acid vulnerability, etc. Another method of lining is disclosed in U.S. Patent No. 4,538,990 which describes applying a 1 to 30% w/v neutral oxalate salt solution, such as dipotassium oxalate to the smear layer and then applying a 0.5 to 3% w/v of an acidic oxalate salt solution such as monopotassium monohydrogen oxalate to the layer. Research has shown poor seal occlusion of the tubules with this method.



U.S. Patent No. 5,296,026 discloses glass phosphate cement compositions and methods for their use as surgical implant materials to fill cavities in bone and canals in teeth. The cement compositions include  $P_2O_5$ , CaO, SrO and  $Na_2O$  in combination with an aqueous liquid with or without therapeutic agents. Mixing the powder and liquid results in a  
5 hardening reactions. When the cement is implanted into hard tissue, it serves as a filler/graft material and along with the release of leachable constituents it can assist in the healing and maintenance of healthy bone.

Various bioactive and biocompatible glasses have been developed as bone replacement materials. Studies have shown that these glasses will induce or aid osteogenesis  
10 in a physiologic systems. Hench et al, *J. Biomed. Mater. Res.* 5:117-141 (1971). The bond developed between the bone and the glass has been demonstrated to be extremely strong and stable. Piotrowski et al., *J. Biomed. Mater. Res.* 9:47-61 (1975). Toxicology evaluation of the glasses has shown no toxic effects in bone or soft tissue in numerous in vitro and in vivo models. Wilson et al., *J. Biomed. Mater. Res.* 805-817 (1981). The glass has been reported  
15 to be bacteriostatic or bacteriocidal most likely related to the change in pH induced by the dissolution of the ions from the surface of the glass and lack of bacterial adherence to the glass surface. Stoor et al, *Bioceramics* Vol. 8 p. 253-258 Wilson et al (1995).

The bonding of the glass to bone begins with the exposure of the glass to aqueous solutions.  $Na^+$  in the glass exchanges with  $H^+$  from the body fluids causing the pH to  
20 increase. Ca and P migrate from the glass forming a Ca-P rich surface layer. Underlying this Ca-P rich is a layer which becomes increasingly silica rich due to the loss of Na, Ca and P ions (U.S. Patent No. 4,851,046).

The behavior of the bioactive glass as solid implants in a dental application was reported by Stanley et al., *Journal of Prosthetic Dentistry*, Vol. 58, pp. 607-613 (1987).  
25 Replicate tooth forms were fabricated and implanted into extracted incisor sockets of adult baboons. Successful attachment of the implants to surrounding bone was seen after histologic examination at six months. Clinical application of this technique is presently available for human use. Endosseous Ridge Maintenance Implant ERMI®. Particulate bioactive glass has been used for periodontal osseous defect repair (US Patent No. 4,851,046) utilizing a size  
30 range of 90-710  $\mu m$  and a compositional range described in the following chart.

## Component Weight Percentage

	SiO <sub>2</sub>	40-55
	CaO	10-30
	Na <sub>2</sub> O	10-35
5	P <sub>2</sub> O <sub>5</sub>	2-8
	CaF <sub>2</sub>	0-25
	B <sub>2</sub> O <sub>3</sub>	0-10

Previously described data has shown that 60% silica is beyond the limit of bioactive melt derived glasses. Okasuki et al. Nippon Seramikbusu Kyokai Gakijutsu Konbuski, Vol. 99, pp. 1-6 (1991).

The 90-710  $\mu$ m size range was determined to be the most effective for periodontal applications when in direct contact with bone. However, size ranges smaller than 90  $\mu$ m were ineffective due to their high rate of reactivity and rapid resorption at the bony site. Moreover, size ranges smaller than 90  $\mu$ m were determined to be ineffective in soft tissue sites also due to the presumption that the smaller particles were removed by macrophages (see U.S. Patent No. 4,851,046). A size range of less than 200  $\mu$ m was also found to be ineffective in certain bone defects (see U.S. Patent No. 5,204,106) due to the high rate of reactivity.

U.S. Patent No. 4,239,113 ("the '113 patent") also describes the use of a bone cement. The '113 patent only discloses bioactive glass ceramic powder having a particle size of 10-200 microns. Moreover, the '113 patent also requires the use of methylmethacrylate (co)polymers and vitreous mineral fibers.

None of the foregoing methods or compositions provide for the combined advantages of both easy application and adherence to tooth structure including penetration into very small tooth structure defects and the opportunity for continued chemical and physical interaction with tooth structure after application.

Accordingly, it is an object of the present invention to provide a composition capable of chemical and physical interaction with tooth structure that is easily applied and readily adherent to tooth structure.

It is a further object of the invention to provide a method of using such a bioactive glass composition to treat a variety of dental and other conditions.

### SUMMARY OF THE INVENTION

5 The present invention relates to, for example, a bioactive glass composition including particulate bioactive and biocompatible glass including by weight percentage:

	SiO <sub>2</sub>	40-60
	CaO	10-30
	Na <sub>2</sub> O	10-35
	P <sub>2</sub> O <sub>5</sub>	2-8
10	CaF <sub>2</sub>	0-25
	B <sub>2</sub> O <sub>3</sub>	0-10
	K <sub>2</sub> O	0-8
	MgO	0-5,

15 the particulate bioactive and biocompatible glass including particles less than 90 µm and an effective remineralizing amount of particles less than about 10 µm. The present invention also relates to various methods of dental treatment including remineralization, sealing fissures and/or pits, lining tooth structure, treating decay, capping pulp, treating sensitive post surgical tooth structure, sealing dentinal tubules, and surface for tissue regeneration.

### 20 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a dentin control surface that has been treated with 37% phosphoric acid for 30 seconds to remove any smear layer after sectioning and grinding to emulate clinical sensitivity. The surface has not been treated with bioactive glass in accordance with the present invention (2000X magnification).

25 Figure 2 is a dentin control surface that has been treated with 37% Phosphoric acid for 30 seconds to remove any smear layer after sectioning and grinding to emulate clinical sensitivity. The surface has not been treated with bioactive glass in accordance with the present invention (3000X magnification).

Figure 3 is a dentin surface that has been treated with an acid etch and treated with a bioactive glass composition in accordance with the present invention in water and glycerin for 2 minutes (Particle size range submicron to 90  $\mu\text{m}$ , 1000X magnification).

5 Figure 4 is a dentin surface that has been acid etched and subsequently treated with a bioactive glass composition in accordance with the present invention in water and glycerin for 2 minutes. The surfaces were subsequently agitated and water rinsed for 2 minutes (Particle size range submicron to 20  $\mu\text{m}$ , 2000X magnification).

10 Figure 5 is a dentin surface that has been acid etched and subsequently treated with a bioactive glass composition in accordance with the present invention and placed in water for 3 days. There was no subsequent agitation, but the surface was water rinsed for 2 minutes (Particle size range submicron to 90  $\mu\text{m}$ , 2000X magnification).

15 Figure 6 is a dentin surface that has been acid etched and subsequently treated with a bioactive glass composition in accordance with the present invention in water and toothpaste for 2 minutes with agitation and a subsequent 2 minute water rinse (Particle size range submicron to 3  $\mu\text{m}$ , 3000X magnification).

Figure 7 is a dentin surface that has been acid etched and treated with a bioactive glass composition in accordance with the present invention in water and toothpaste for 2 minutes with agitation and water rinse for 2 minutes (Particle size range submicron to 3  $\mu\text{m}$ , 3500X magnification).

20 Figures 8 and 9 each include a dentin surface which has been acid etched with phosphoric acid, treated with a bioactive glass in accordance with the present invention for 2 minutes and immersed in a phosphate buffered saline for 5 days (Particle size range submicron).

25 Figure 10 depicts a dentin surface that has been acid etched and subsequently treated with a single application of a bioactive glass composition in accordance with the present invention.

Figure 11 depicts a dentin surface that has been acid etched and treated with three separate applications of a bioactive glass composition in accordance with the present invention.

30 Figure 12 is a Fourier Transform Spectroscopy (FTIR) performed on samples treated with optimal sizes and shaped particulate bioactive glass.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a bioactive glass composition which is useful in, for example, enamel remineralization, incipient caries remineralization, carious dentin  
5 remineralization, caries prevention, arresting decay, reversing decay, anti-caries, pit and fissure sealants, prophylactic pastes, fluoride treatments, dentinal sealants, etc. It can also be included in toothpastes, liners, bases, gels, and restorative material e.g. packing, indirect pulp capping agent, etc. Compositions in accordance with the present invention are also useful in the treatment of surfaces after periodontal surgery to decrease dentinal sensitivity and  
10 enhance tissue attachment. The compositions are active in treating various defects associated with a variety of dental and other conditions and actually chemically and physically bond to the tooth thereby remineralizing tooth structure.

As referred to herein, remineralization is the formation of hydroxyapatite. The formation of hydroxyapatite begins with exposure of a bioactive glass composition to  
15 aqueous solutions. It is believed that the sodium ions ( $\text{Na}^+$ ) in the bioactive glass exchanges with  $\text{H}^+$  ions in body fluids causing pH to increase. Calcium and phosphorus then migrate from the bioactive glass forming a calcium-phosphorous rich surface layer. An underlying silica rich zone slowly increases as the sodium ion in the bioactive glass continues to exchange with the hydrogen ion of the solution. After time, the calcium-phosphorous rich  
20 layer crystallizes into a hydroxyapatite material. Collagen can become structurally integrated with the apatite agglomerates. As hereinafter referred to, an effective remineralizing amount is any amount capable of forming hydroxyapatite.

As the term "a tooth structure" is used herein, it is intended to refer to any feature or features of a tooth including but not limited to enamel, dentin, pulp, tooth root structure,  
25 cementum, root dentin, coronal dentin, any dental manufacture, etc.

A bioactive glass in accordance with the present invention is a glass composition that will form a layer of hydroxycarbonate apatite *in vitro* when placed in a simulated body fluid. For example, the following composition by weight will provide a bioactive glass:

	SiO <sub>2</sub>	40-60
	CaO	10-30
	Na <sub>2</sub> O	10-35
	P <sub>2</sub> O <sub>5</sub>	2-8
5	CaF <sub>2</sub>	0-25
	B <sub>2</sub> O <sub>3</sub>	0-10
	K <sub>2</sub> O	0-8
	MgO	0-5

10 Bioactive glasses with these properties provide a more efficacious material for interaction with the tooth structure. A biocompatible glass in accordance with the present invention is one that does not trigger an overwhelmingly adverse immune response.

In accordance with the present invention, it has been found that bioactive glasses of specified particle sizes are particularly useful in treating the above-mentioned conditions. Specifically, surprising results are obtained by the present invention where small and very

15 small particles are combined. For example, when compositions including small particles that are capable of bonding with tooth structure (e.g. less than about 90 microns) as well smaller particles (e.g. less than about 10) are used in combination, the larger of these particles adhere to tooth structure and act as ionic reservoirs while the smaller are capable of entering and lodging inside of various tooth structure surface irregularities. The larger of these particles

20 provide a reservoir of additional calcium and phosphorous so that the mineralization, or depositing of the calcium phosphate layer begun by the small particles can continue. Additional calcium and phosphorous can be leached to all tooth structure as well as to particles which have become attached to the inside or at the openings of surface irregularities of tooth structure such as dentinal tubules. This in turn provides for continuation of the entire

25 reaction and continued growth of the smaller of these particles which have lodged inside or over the openings of such surface irregularities and can result in effectively coating or filling the surface irregularity. This excess concentration of ions of calcium and phosphorous is necessary for continued reaction of the smaller of these particles to take place because the smaller particles quickly exhaust their ions as a result of their relatively high surface area.

30 The larger of these particles will react and release their ions more slowly as a longer term effect. Furthermore, the larger of these particles will mechanically abrade the tooth surface

opening various surface irregularities allowing small particles to enter and react with the surface irregularity.

This effect is very beneficial in a variety of applications. For example, in preventing caries or decay, the composition of the present invention is capable of penetrating into the depths of the smallest of surface irregularities and receiving a continued supply of ions from larger nearby particles so that it is able to grow after exhausting its stored ion supply. This is also very useful in sealing pits and fissures and a much more effective and long lasting seal is obtained.

In some embodiments of the present invention, extremely small particles are used. For example, particles that are in the range of 2  $\mu\text{m}$  to submicron fit inside dentin tubules that are approximately 1-2  $\mu\text{m}$  in diameter. The occlusion of these tubules leads to a significant reduction in the amount of sensitivity after, for example, periodontal surgery. Preferably, a mixture of particles less than two microns and larger than 45 microns in diameter are used. It has been found that this combination yields a particularly effective composition.

Compositions in accordance with the present invention generally do not require time to set. Previous compositions were easily washed away by mechanical abrasion caused by brushing, exposure to mild acids in food, salivary flow or other liquids which normally come in contact with the teeth. However, some compositions in accordance with the present invention have been able to generally withstand significant agitation, rinsing with water and long term soaking in simulated saliva for five days. Moreover, many of the small particles of the present invention do not require a set time because they begin to chemically react and adhere to tooth structure as soon as they come into contact with these surfaces and fluids naturally present in the mouth. Although compositions in accordance with the present invention are effective with a single application, it is likely that multiple applications will be more efficacious.

Surprisingly, the relatively small bioactive particulate glass of the present invention does not generate a significant immune response. Moreover, it is generally not engulfed by macrophages and rendered inactive in this application.

The composition of the present invention is capable of providing a bioactive layer that will form a new structural layer which is a lasting remineralization of tooth structure. This has been verified by the reformation of a hydroxycarbonate apatite layer on dentin surfaces

after treatment with compositions in accordance with the present invention with Fourier Transform Infrared spectroscopy (FTIR).

In one embodiment in accordance with the present invention, the particles have a particle size of about 20 microns with about 30 percent of the particles less than 10 microns.

5 In another embodiment in accordance with the present invention the particles have an average particle size of 10 microns with at least 25% smaller than 2 microns.

The compositions of the present invention may be formulated into toothpaste. In fact, the particles may replace the silica currently used in toothpastes. The addition of fluoride in the glass composition will enhance and strengthen the tooth structure. In addition to direct  
10 application of the bioactive glass to the teeth, the bioactive glass composition of the present invention can also be applied in a saline or distilled water based medium.

The compositions of the present invention may also be formulated into mouthwash, gel or they may be applied by a dentist as a paste.

15

### Examples

The following working examples are non-limiting:

*In vitro* experiments were performed using a standardized slab of human tooth dentin from extracted teeth. These discs were cut from the extracted teeth using an Isomet diamond saw (Buchler Ltd.). The discs were 1.0 mm thick and the size of the tooth. The occlusal  
20 surfaces were ground on a series of wet silicon-carbide papers ranging from 320 to 600 grit. This was done to standardize the test surfaces. The surfaces were treated with 37% phosphoric acid for 60 seconds to remove the smear layer created during the grinding process and open and enlarge all the dentin tubules (See Figures 1 and 2). The surface was rinsed with distilled water for 20 seconds and dried with a stream of oil free air. Each slab was split  
25 in half and the experimental material placed on one-half of the specimen as described in the examples. An untreated slab with open and enlarged tubules is shown in Figures 1 and 2.

Scanning electron microscopy was performed on the slab surface in each group. The slabs were mounted on scanning electron microscope stubs using sliver paste. All specimens were vacuum dried, sputter coated and examined in a JEOL-T200 scanning electron  
30 microscope.



EXAMPLE 1

The starting product was a mixture containing (% by weight)

	SiO <sub>2</sub>	45
5	CaO	24.5
	Na <sub>2</sub> O	24.5
	P <sub>2</sub> O <sub>5</sub>	6

The mixture was melted in a covered platinum crucible at 1350° C for 2 hours to achieve homogenization. The mixture was later quenched in deionized water at 0°C. Fritted glass  
10 was placed in an appropriate milling apparatus including ball mill, impact mill. The glass is milled for 2 hours and separated into appropriate size ranges.

The particle size range less than 90 µm was obtained using this process and confirmed by scanning electron microscopy and laser light scattering technique (Coulter LS 100). These mixtures were placed on the dentin slabs previously described.

15 The exposure times to the dentin varied between two minutes with scrubbing to 3 days with no agitation. The occlusion of the tubules is depicted in Figures 3-7. Visible in Figures 3-7 are total and partial occlusion of the dentin tubules with multiple size of small (1-5 µm) particles present. In addition, larger particles that are visible that will act as reservoirs for the chemical composition. Early formation of hydroxyapatite crystals is beginning on the  
20 dentin surface confirmed by FTIR.

EXAMPLE 2

Figures 8 and 9 indicate the results obtainable by using submicron particles made in  
25 accordance with Example 1. The samples of figures 8 and 9 are dentin surfaces which have been acid etched with phosphoric acid, treated with a bioactive glass for 2 minutes and immersed in a phosphate buffered saline for 5 days. With the lack of large particles for reservoir activity, there was less complete regeneration as confirmed by FTIR.

### EXAMPLE 3

Example 3 was conducted to illustrate the benefits associated with multiple applications of compositions in accordance with the present invention. First, an acid etched dentin surface was treated with a single treatment of bioactive particulate glass for two minutes and is depicted in Figure 10. A dentin surface which has been acid etched and treated three times for two minutes is depicted in Figure 11.

Figure 10 shows significant penetration and occlusion of the tubules with a bonding over the surface of the dentin. There are not many large particles visible in Figure 10. In Figure 11, there is even more significant penetration and occlusion of the tubules and a greater number of particles present. This demonstrates the benefits associated with multiple application including the tubules as well as increased presence of larger reservoirs of Ca and P ions. This also demonstrates interparticle welding of the larger particles to the smaller particles already bound to the surface.

### EXAMPLE 4

Example 4 further illustrates the benefits associated with the use of particles less than 2 microns in combination particles greater than 45 microns in size. FTIR spectra for the following samples are included in figure 12 to illustrate remineralization:

Sample No. 1	Control (untreated dentin surface)
Sample No. 2	Acid etched dentin surface
Sample No. 3	Treated with particles of bioactive glass less than 2 microns in particle size for two minutes
Sample No. 4	Treated with particles of bioactive glass wherein 40% were less than 2 microns, 15% were in the range of 8 to 2 microns, 15% were in the range of 8 to 20 microns, 15% were in the range of 20 to 38 microns and 15% were in the range of 38-90 microns.

As illustrated in Figure 12, the control sample provides a representative view of the spectrum of hydroxycarbonate apatite (HCA). The shape of the peaks between wave number

1150 to 500 are very characteristic of HCA. In sample 2, the peaks are disrupted after treatment with the acid etchant, especially in the 1150 to 900 range. This indicates a loss of the mineral components of the tooth structure, Calcium and Phosphorous. Sample 3 shows a partial remineralization of the Ca and P on the tooth structure. Sample 4 was treated with the optimal size and shape mixture of bioactive glass and shows an almost complete remineralization. A photomicrograph of Sample 4 is included as Figure 11.

### EXAMPLE 5

Comparative Example 5 shows the benefits associated with the use of particles less than 10 microns in combination with particles greater than 45 microns in size over the use of just particles less than 2 microns or 53-90 $\mu$ . A control sample of untreated dentin surface was used in addition to treated surfaces as described below:

Number of Applications	Sample Composition	Score	Observations
Single	53-90 $\mu$	2	About 50% occluded tubules with large particles present
	Control	0	No particles present
Single	<2 $\mu$	2	Above 50% closure, no large particles seen
	Control	0	Open tubules
Single	50% 53-90 $\mu$ 50% <2 $\mu$	+3	75%+ tubules occluded
	Control	0	Open tubules
Multiple	53-90 $\mu$	2	Partial closure of tubules with large particles present
	Control	0	Minimal occlusion seen

Multiple	<2 $\mu$	2	Partial closure of tubules with small particles present
	Control	0	Minimal occlusion seen
Multiple	50% 53-90 $\mu$ 50% <2 $\mu$	+3	Best results-- tubules closed; difficult to find open tubules
	Control	0	Minimal occlusion seen

5 All samples in the above Table were subjected to a moist environment for 24 hours and then dried for 48 hours.

As seen above, the combination of particles less than 2 microns and 53-90 $\mu$  provided the best results. It is believed that the presence of both size ranges permits the smaller particles which have lodged in the tubules to continue growth after they have exhausted their own Ca and P ions and are able to make use of such ions from other nearby larger particles acting as reservoirs of Ca and P ions.

#### OTHER EXAMPLES

15 The composition of the starting product for the following examples was the same as Example 1 except the level of SiO<sub>2</sub> was 45%, 55%, and 60%. Also, the method of preparation was different. The mixture was melted in a covered platinum crucible at 1350°C for 2 hours to achieve homogenization. The mixture was poured into a slab, allowed to cool to room temperature and crushed with a hammer. Crushed glass fractions were then separated by sieving through a standard screen. Fractions were then separated and retained.

20 The particle size range less than 90  $\mu$ m was obtained using this process and confirmed by scanning electron microscopy and laser light scattering technique (Coulter LS 100). These mixtures were placed on the dentin slabs previously described.

25 Samples containing 45%, 55%, and 60% SiO<sub>2</sub> were utilized in the preparations with the same results seen in Example 1. Again, the key to this data was the presence of the size range of particles. Present in these examples are ranges up to 60% silica with a size range in

particles from submicron to 90 micron showing like reactions to Example 1 on the dentin surfaces.

Although the present invention has been described in one or more embodiments, this description is not intended to in any way limit the scope of the claims.

We claim:

1. A bioactive glass composition comprising particulate bioactive and biocompatible glass including by weight percentage:

5	SiO <sub>2</sub>	40-60
	CaO	10-30
	Na <sub>2</sub> O	10-35
	P <sub>2</sub> O <sub>5</sub>	2-8
	CaF <sub>2</sub>	0-25
	B <sub>2</sub> O <sub>3</sub>	0-10
10	K <sub>2</sub> O	0-8
	MgO	0-5,

the particulate bioactive and biocompatible glass including particles less than 90 µm and an effective remineralizing amount of particles less than about 10 µm.

2. A method for preventing tooth decay comprising contacting a tooth structure with the composition of claim 1.

3. A method for treating tooth decay comprising contacting a tooth structure with the composition of claim 1.

4. A method for preventing incipient caries comprising contacting a tooth structure with the composition of claim 1.

5. A method for remineralizing enamel comprising contacting a tooth structure with the composition of claim 1.

6. A method for incipient caries remineralization comprising contacting a tooth structure with the composition of claim 1.

7. A method for sealing fissures in tooth structure comprising contacting a tooth structure with the composition of claim 1.

8. A method for sealing pits in tooth structure comprising contacting a tooth structure with the composition of claim 1.

9. A method for lining tooth structure comprising contacting a tooth structure with the composition of claim 1.

10. A method for capping pulp comprising contacting a tooth structure with the composition of claim 1.

11. A method for treating tooth hypersensitivity comprising contacting a tooth structure with the composition of claim 1.

12. A method for treating tooth structure after periodontal surgery comprising contacting a tooth structure with the composition of claim 1.

13. A composition for treating teeth comprising the composition of claim 1 and a toothpaste, liner, base, gel, restorative material, glycerin gel, mouthwash, prophylactic paste, or indirect pulp capping agent, or mixture thereof.

14. A bioactive glass composition comprising particulate bioactive and biocompatible glass including by weight percentage:

SiO <sub>2</sub>	40-60
CaO	10-30
Na <sub>2</sub> O	10-35
P <sub>2</sub> O <sub>5</sub>	2-8
CaF <sub>2</sub>	0-25
B <sub>2</sub> O <sub>3</sub>	0-10
K <sub>2</sub> O	0-8
MgO	0-5,

the particulate bioactive and biocompatible glass including particles between 45μ and 90 μm and an effective remineralizing amount of particles less than about 10 μm.

15. A method for preventing tooth decay comprising contacting a tooth structure with the composition of claim 14.

16. A method for treating tooth decay comprising contacting a tooth structure with the composition of claim 14.

17. A method for preventing incipient carries comprising contacting a tooth structure with the composition of claim 14.

18. A method for remineralizing enamel comprising contacting a tooth structure with the composition of claim 14.

19. A method for incipient caries remineralization comprising contacting a tooth structure with the composition of claim 14.

20. A method for sealing fissures in tooth structure comprising contacting a tooth structure with the composition of claim 14.

21. A method for sealing pits in tooth structure comprising contacting a tooth structure with the composition of claim 14.

22. A method for lining tooth structure comprising contacting a tooth structure with the composition of claim 14.

23. A method for capping pulp comprising contacting a tooth structure with the composition of claim 14.

24. A method for treating tooth hypersensitivity comprising contacting a tooth structure with the composition of claim 14.

5 25. A method for treating tooth structure after periodontal surgery comprising contacting a tooth structure with the composition of claim 14.

26. A composition for treating teeth comprising the composition of claim 14 and a toothpaste, liner, base, gel, restorative material, glycerin gel, mouthwash, prophylactic paste, or indirect pulp capping agent, or mixture thereof.

10 27. A bioactive glass composition comprising particulate bioactive and biocompatible glass including particles less than 90  $\mu\text{m}$  and an effective remineralizing amount of particles less than about 10  $\mu\text{m}$ .

28. A bioactive glass composition comprising particulate bioactive and biocompatible glass including particles less than 90  $\mu\text{m}$  and an effective remineralizing  
15 amount of particles less than about 5  $\mu\text{m}$ .

29. A bioactive glass composition comprising particulate bioactive and biocompatible glass including particles less than 90  $\mu\text{m}$  and an effective remineralizing amount of particles less than about 2  $\mu\text{m}$ .

30. A method for preventing tooth decay comprising contacting a tooth structure  
20 with the composition of claim 27.

31. A method for treating tooth decay comprising contacting a tooth structure with the composition of claim 27.

32. A method for preventing incipient carries comprising contacting a tooth structure with the composition of claim 27.

25 33. A method for remineralizing enamel comprising contacting a tooth structure with the composition of claim 27.

34. A method for incipient caries remineralization comprising contacting a tooth structure with the composition of claim 27.

30 35. A method for sealing fissures in tooth structure comprising contacting a tooth structure with the composition of claim 27.



36. A method for sealing pits in tooth structure comprising contacting a tooth structure with the composition of claim 27.

37. A method for lining tooth structure comprising contacting a tooth structure with the composition of claim 27.

5 38. A method for capping pulp comprising contacting a tooth structure with the composition of claim 27.

39. A method for treating tooth hypersensitivity comprising contacting a tooth structure with the composition of claim 27.

10 40. A method for treating tooth structure after periodontal surgery comprising contacting a tooth structure with the composition of claim 27.

41. A composition for treating teeth comprising the composition of claim 27 and a toothpaste, liner, base, gel, restorative material, glycerin gel, mouthwash, prophylactic paste, or indirect pulp capping agent, or mixture thereof.

15 42. A bioactive glass composition comprising particulate bioactive and biocompatible glass including particles less than 90  $\mu\text{m}$  and particles less than about 2  $\mu\text{m}$ .

43. A method for remineralizing tooth structure comprising contacting a tooth structure in need of remineralization with a bioactive glass composition including an effective remineralizing amount of particles less than 90  $\mu\text{m}$ .

20 44. A bioactive glass composition comprising particulate bioactive and biocompatible glass including by weight percentage:

	$\text{SiO}_2$	40-60
	$\text{CaO}$	10-30
	$\text{Na}_2\text{O}$	10-35
	$\text{P}_2\text{O}_5$	2-8
25	$\text{CaF}_2$	0-25
	$\text{B}_2\text{O}_3$	0-10
	$\text{K}_2\text{O}$	0-8
	$\text{MgO}$	0-5,

the particulate bioactive and biocompatible glass including particles between 53 $\mu$  and 90  $\mu\text{m}$  and an effective remineralizing amount of particles less than about 2  $\mu\text{m}$ .

30

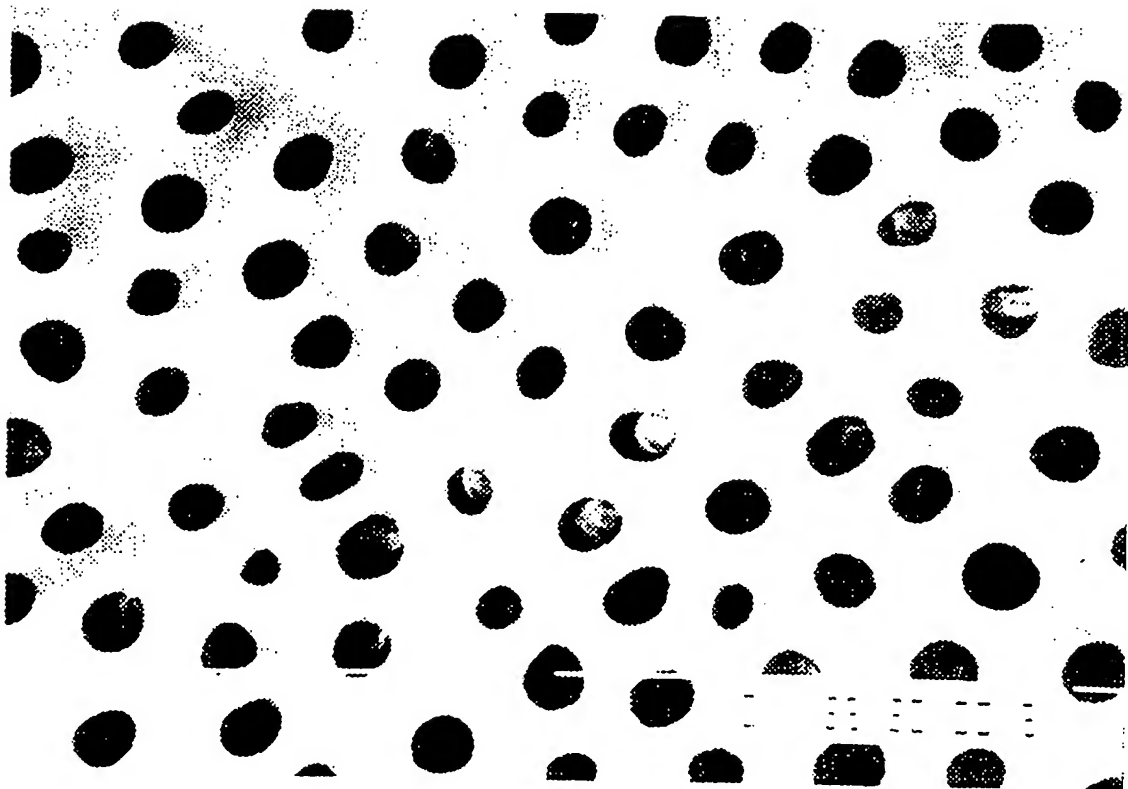


FIG. 1

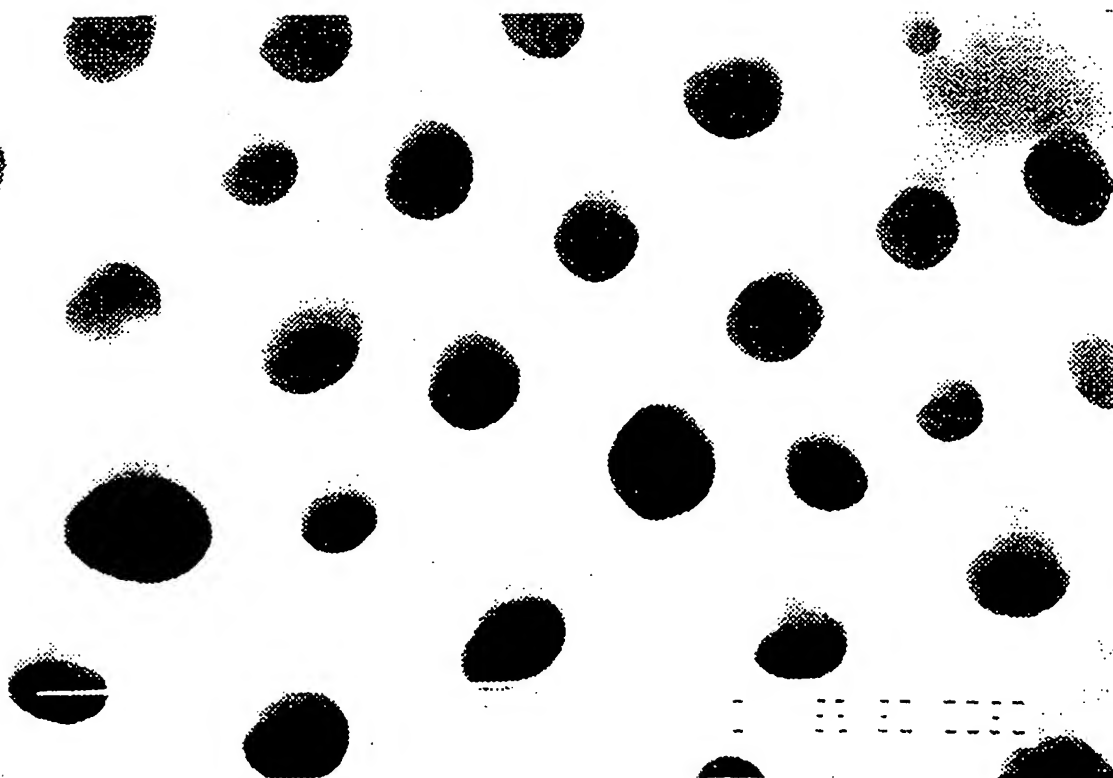


FIG. 2



FIG. 3

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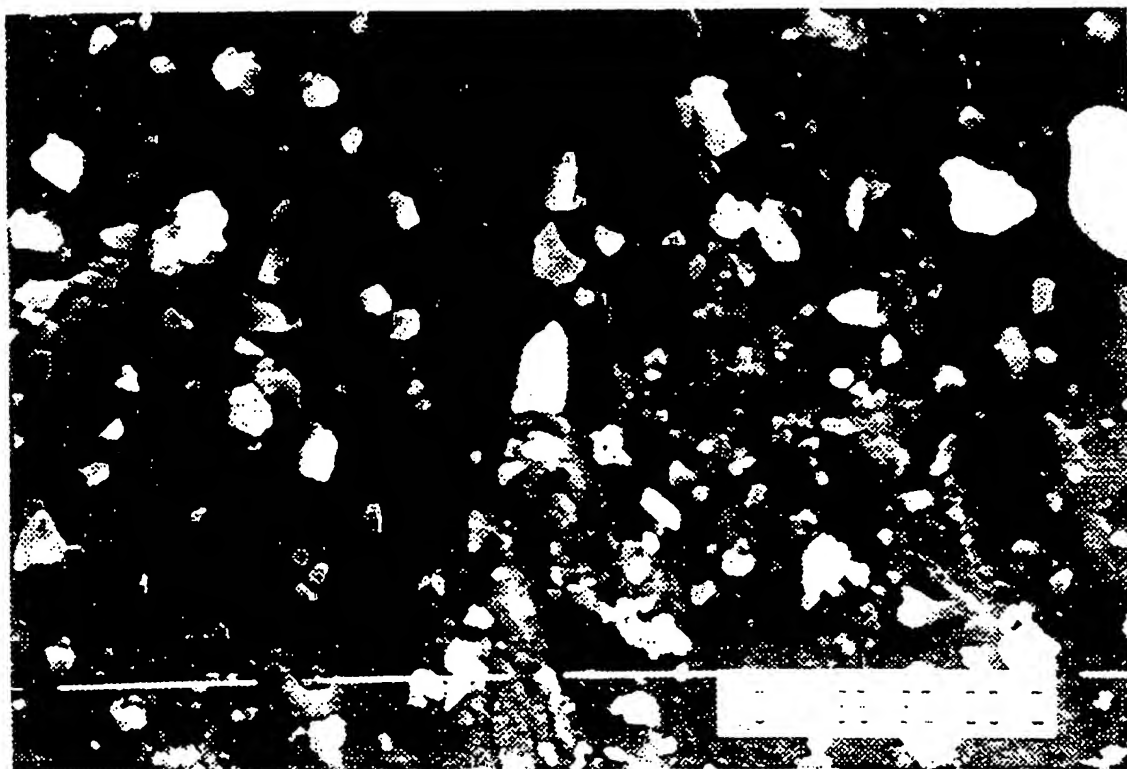


FIG. 4

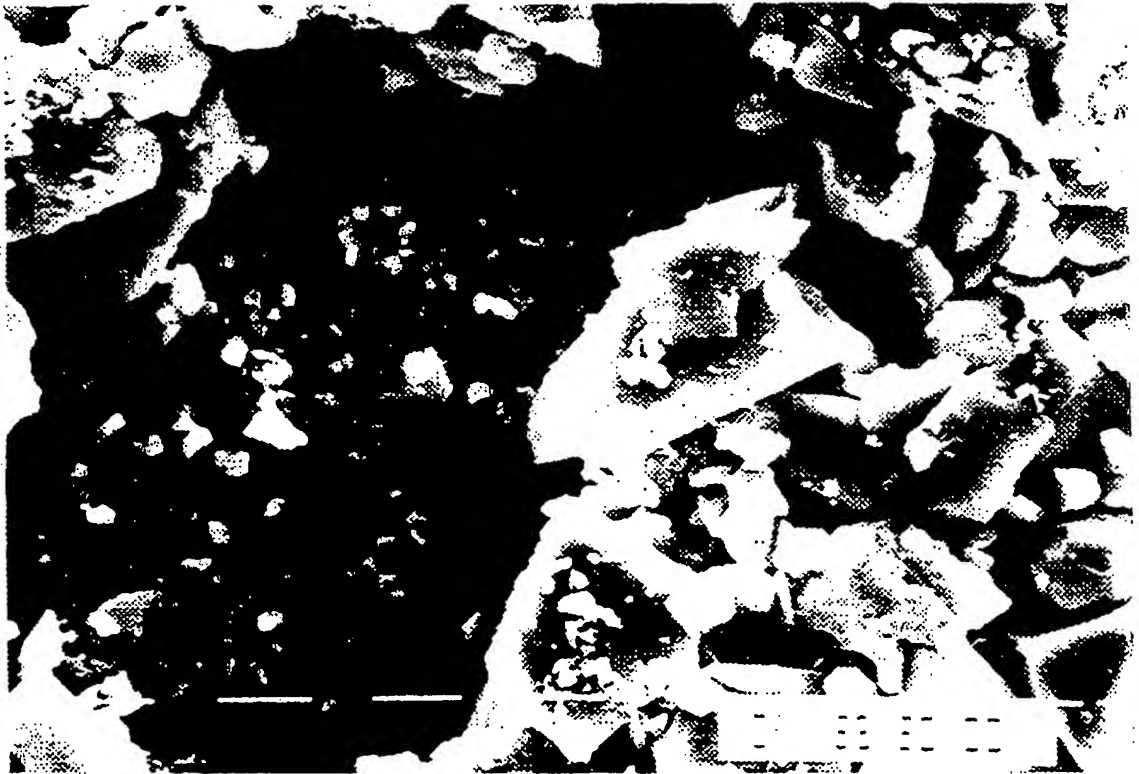


FIG. 5

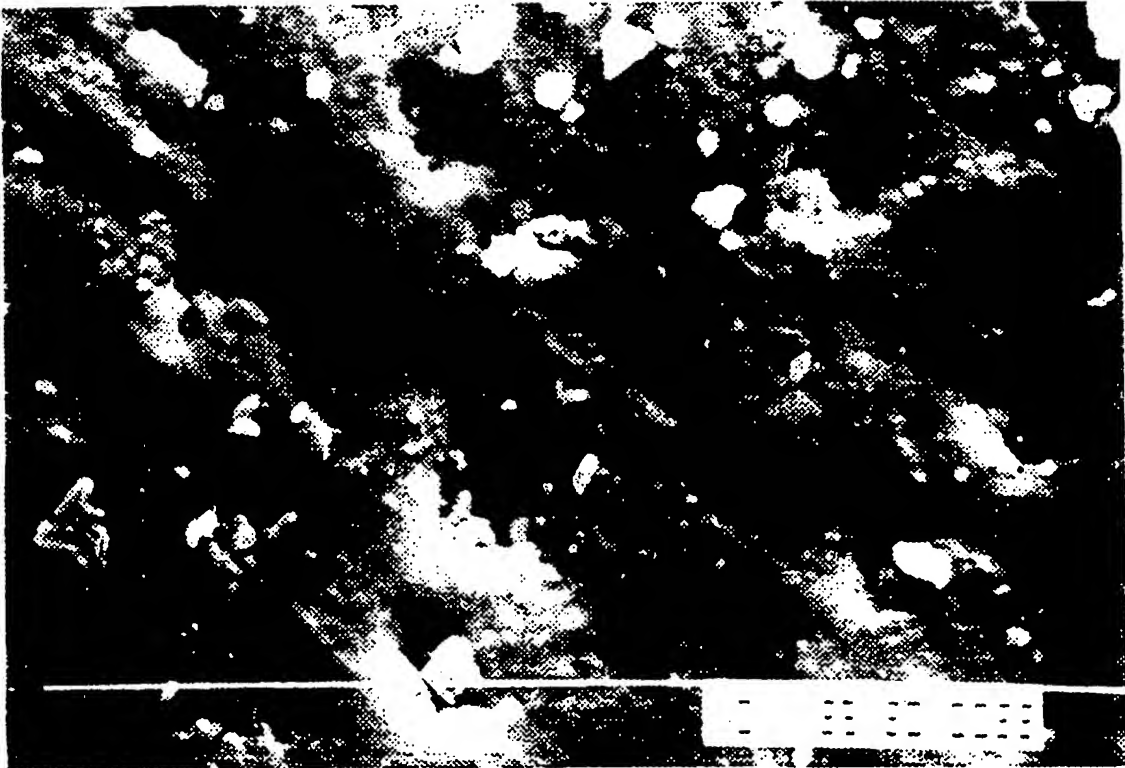


FIG. 6

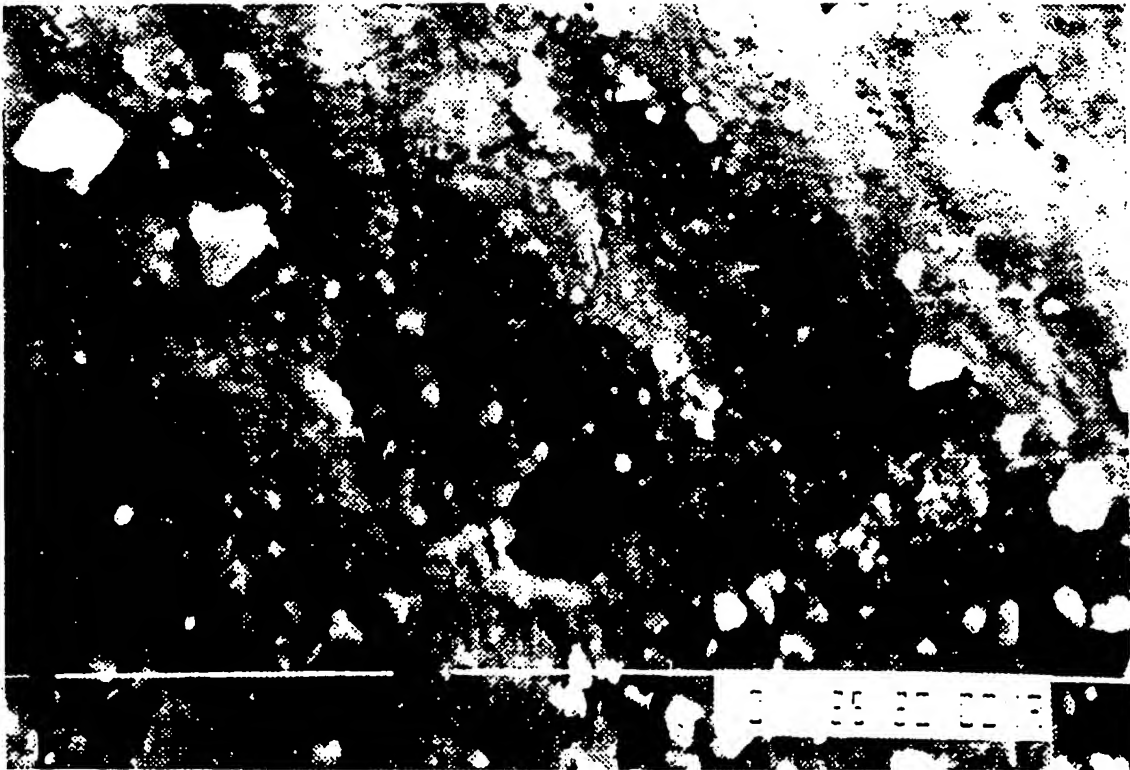


FIG. 7



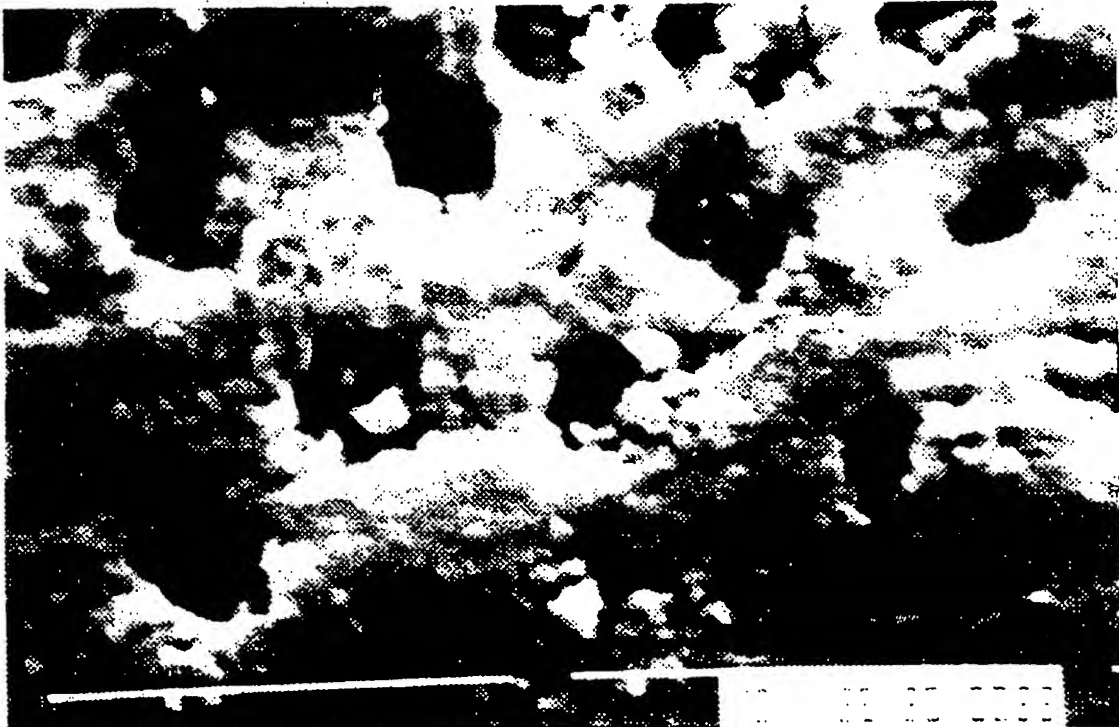


FIG. 8

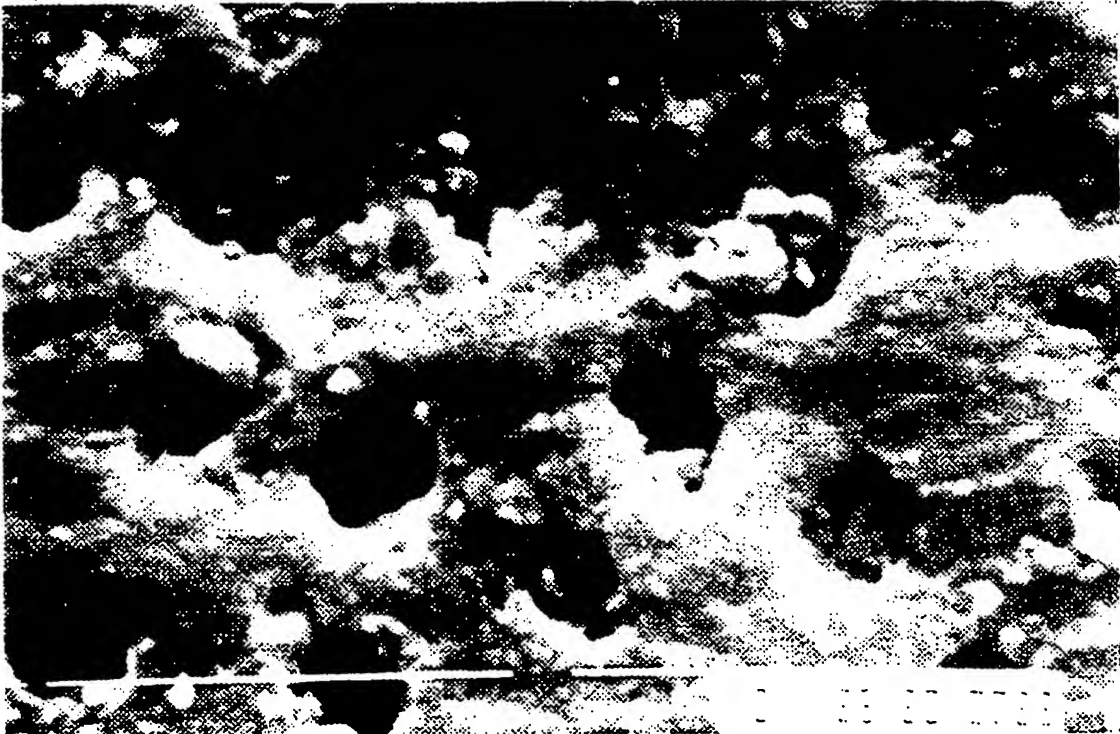


FIG. 9

10/12

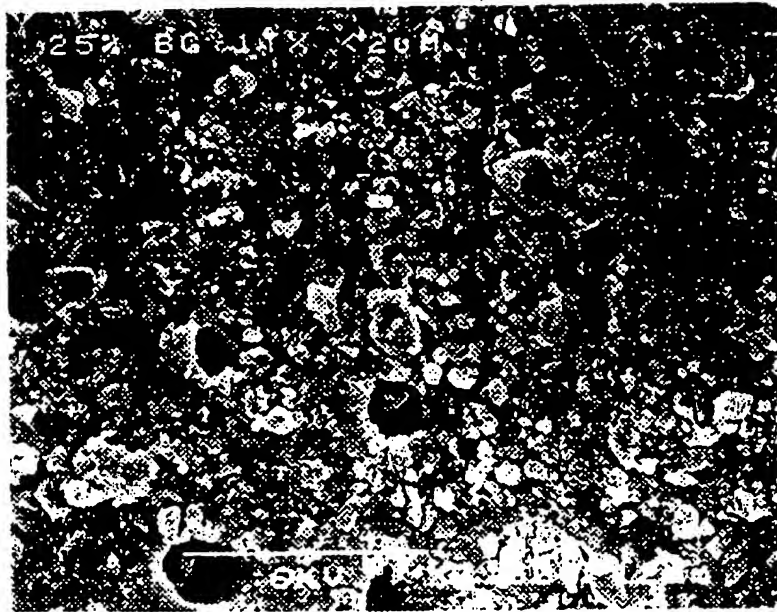


FIG. 10



FIG. 11

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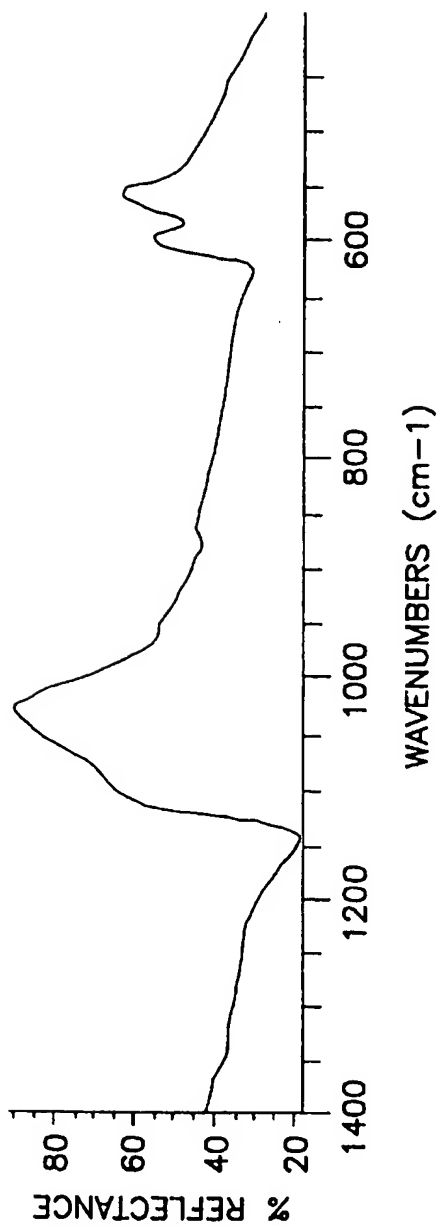


FIG. 12A

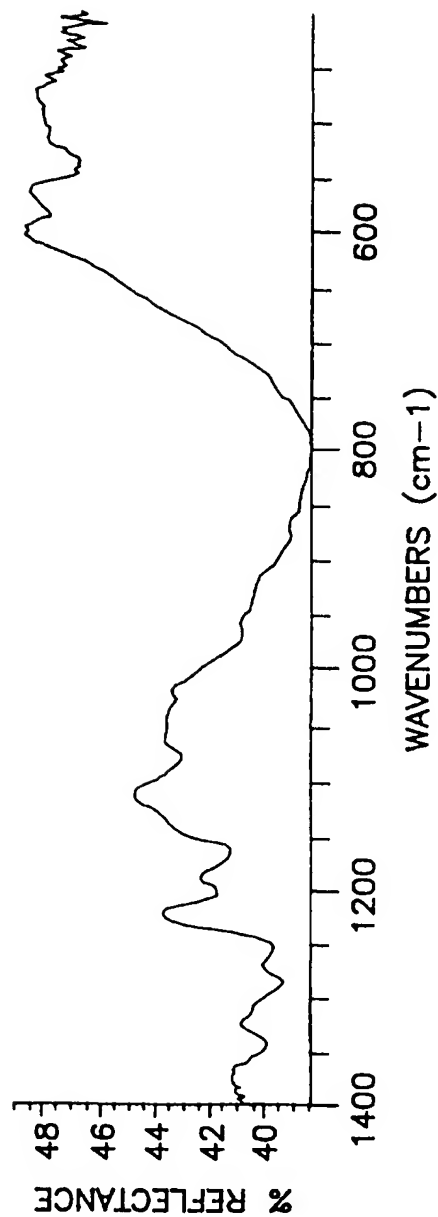
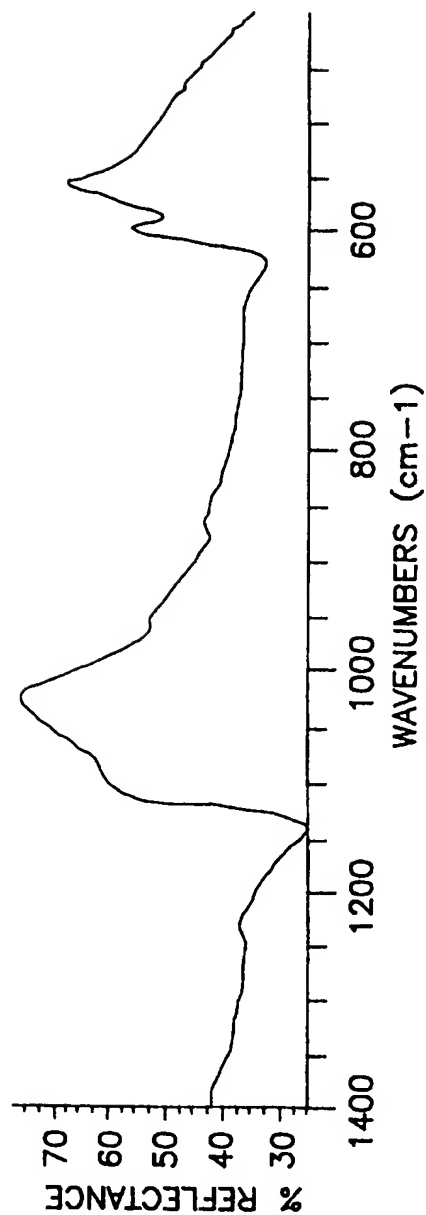
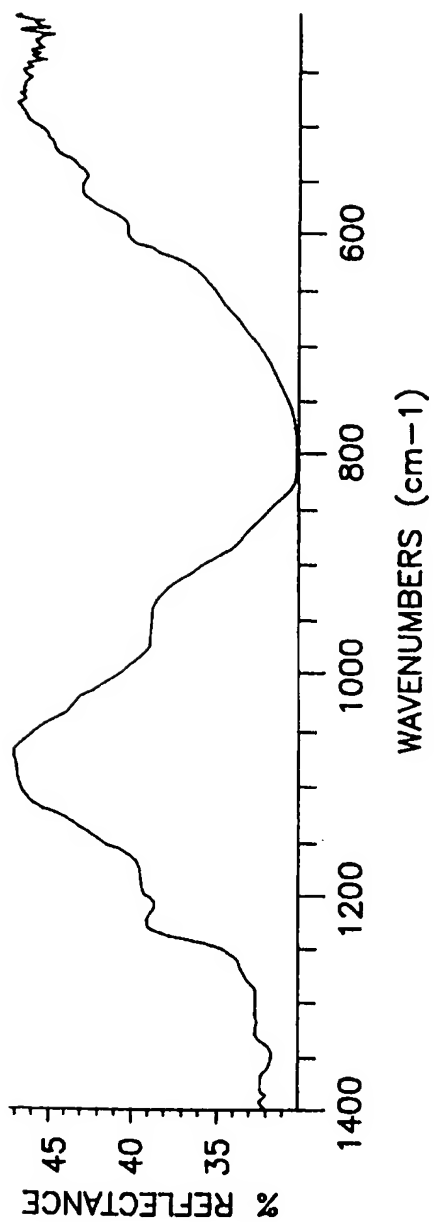


FIG. 12B



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/01785

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : CO3C 3/078; A61K 6/027

US CL : 106/35; 501/24, 25, 57, 58, 63, 72; 424/49, 52, 57; 433/226

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 106/35; 501/24, 25, 57, 58, 63, 72; 424/49, 52, 57; 433/226

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,605,415 A (RICHEZ) 12 August 1986, col. 4, lines 47-60.	27-43
X	US 4,783,429 A (SHIBUYA ET AL) 08 November 1988, col. 4, lines 38-39.	27-43
X	US 4,775,592 A (AKAHANE ET AL) 04 October 1988, col. 3, lines 29-36.	27-43
A	US 5,204,106 A (SCHEPERS ET AL) 20 April 1993, col. 3, lines 25-38.	1-44
A	US 4,851,046 A (LOW ET AL) 25 JULY 1989, col. 6, lines 35-43.	1-43
A	US 4,057,621 A (PASHLEY ET AL) 08 November 1977, entire document.	1-43

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

Special categories of cited documents:	
*A* document defining the general state of the art which is not considered to be of particular relevance	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*E* earlier document published on or after the international filing date	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O* document referring to an oral disclosure, use, exhibition or other means	*A* document member of the same patent family
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

25 MARCH 1997

Date of mailing of the international search report

08 MAY 1997

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 Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

KARL GROUP

Telephone No. (703) 308-0661

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/01785

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,239,113 A (GROSS ET AL) 16 December 1980, entire document.	1-43
A	US 5,120,340 A (DUCHEYNE ET AL) 09 June 1992, col. 3, lines 24-62.	1-43
A	US 5,429,996 A (KANEKO) 4 July 1995, entire document.	1-43